SCIENTIFIC VALIDATION OF SIDDHA POLY- HERBAL FORMULATION "*THIRIJADHATHI MATHIRAI*" FOR ITS ANTI-TUBERCULOSIS, BRONCHODILATOR AND ANTI-HISTAMINE ACTIVITY IN ANIMAL MODEL.

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ARUMBAKKAM, CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation of Siddha Poly - herbal formulation *Thirijadhathi Mathirai* For its Anti-tuberculosis, Bronchodilator and Anti-histamine Activity in Animal model"** is a Bonafide and genuine research work carried out by me under the guidance of **Dr.R. Karolin Daisy Rani M.D(S)., Lecturer,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation of Siddha Poly- herbal formulation *Thirijadhathi Mathirai* For its Anti-tuberculosis, Bronchodilator and Anti-histamine Activity in Animal model"** is a Bonafide work carried out by **Dr. S. Poonkuzhali** under the guidance of **Dr. Dr.R. Karolin Daisy Rani, M.D(s)., Lecturer,** Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Chennai - 106.

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ABBREVIATIONS

AIDS	**Acquired Immuno Deficiency Syndrome**
AMK	**Amikacin**
AST	**Aspartate transaminase**
ANOVA	**Analysis Of Variance**
Bcl 2	**B Cell lymphoma 2**
BUN	**Blood Urea Nitrogen**
CPCSEA	**Committee for the Purpose of Control and Supervision of Experimental Animals.**
CMC	**Carboxymethyl Cellulose**
CFA	**Component of Freund's complete Adjuvant**
CLA	**Conjugated Linolenic Acid**
DMSO	**Dimethyl sulfoxide**
EDTA	**Ethylene Diamine Tetra Acetic acid**
EUS	**Endoscopic Ultrasound Needle aspiration**
FDA	**Food and Drug Administration**
FTIR	**Fourier Transform Infrared Spectrometry**
GOT	**Glutamate Oxaloacetate transaminase**
GPT	**Glutamate Pyruvate Transaminase**
HDL	**High Density Lipoprotein**
HIV	**Human Immuno deficiency virus**
HL-60	**Human promyelocytic Leukaemia cells**
HPTLC	**High Performance Thin Layer Chromatography**

ICMR	**Indian Council of Medical Research**
ICPOES	**Inductively Coupled Plasma Optical Emission Spectrometry**
IAEC	**Institutional Animal Ethical Committee**
IP	**Indian Pharmacopoeia**
KM	**Kanamycin**
LDL	**Low Density Lipoprotein**
LJ medium	**Lowenstein – Jensen medium**
LTD4	**Leukotrienes D4**
LTC4	**Leukotrienes C4**
LPD	**Lipoamide dehydrogenase**
LRP	**Luciferase reporter phage**
MIC	**Minimum Inhibitory Concentration**
MIR	**Medical International Research**
MP	**Mycobacteriophage buffer**
MTP	**Media Transfer Protocol**
MCV	**Mean corpuscular volume**
MDR-TB	**Multi- drug Resistant Tuberculosis**
NIRT	**National Institute for Research in Tuberculosis**
NSAIDS	**Non-Steroidal Anti Inflammatory Drug**
OECD	**Organisation for economic corporation and development**

PCR	**Polymerase Chain Reaction**
PDH	**Pyruvate Dehydrogenase Complex**
PCT	**Pro Convulsion Time**
PCV	**Packed Cell Volume**
PET	**Positron Emission Tomography**
PNR/P	**Peroxy Nitrite Reductase/ Peroxidase**
RBC	**Red Blood Corpuscles**
RLU	**Relative Light Units**
ROS	**Robot Operating System**
SCMC	**Standard Carboxyl Methyl Cellulose**
SEM	**Scanning Electron Microscope**
SEM	**Standard Error Mean**
WBC	**White Blood Corpuscles**
WHO	**World Health Organization**
XDR-TB	**Extensively Drug Resistant- Tuberculosis**

INTRODUCTION

" உற்றவன் தீர்ப்பான் மருந்துழைச் செல்வானென்று
அப்பால்நாற் கூற்றே மருந்து"[1]

- திருக்குறள்- எண். 950

"HEALTH IS NOT VALID TILL SICKNESS COMES"

Tuberculosis is a communicable chronic granulomatous disease caused by *Mycobacterium tuberculosis*. It usually involves the lungs but may affect any organ or tissue in the body, but the pulmonary system is the primary target for *Mycobacterium tuberculosis*.

Anti-tuberculosis agents are drugs with different mechanism of action having activity against *Mycobacterium tuberculosis*.

Tuberculosis accounts for millions of death worldwide every year. Diverse survival strategies adopted by *Mycobacterium tuberculosis* have substantially hindered the existing anti-TB regimen thereby leading to Multi- Drug Resistant (MDR) and Extremely Drug Resistant (XDR) strains of tuberculosis.[1]

"THE ART OF MEDICINE CONSISTS IN AMUSING THE PATIENT WHILE NATURE CURES THE DISEASE."

Keeping the aforesaid facts in view, medicinal plants may represent a potential option to combat the threat of Tuberculosis. The trial drug, an herbal formulation, ***"THIRIJADHATHI MATHIRAI"*** will be a new and effective alternative medicine for tuberculosis.

The respiratory system is an outgrowth from the ventral wall of the foregut.[2] The lungs are a pair of spongy, air filled, respiratory organ situated in the thoracic cavity. The lungs are lined by epithelial cells, which help trap particles and prevent infection from reaching the lungs.

Important functions of the lungs are,

1. Gaseous exchange
2. Removal of waste and toxins
3. Defence against infection and harmful substance

The airways are the central component of the lung, organised in a bronchial network. It is an immunological interface under constant assault by inhaled particulates and pathogens.[1]

"NATURAL FORCES WITHIN US ARE
THE TRUE HEALERS OF DISEASE"
- HIPPOCRATES

Lung diseases, particularly tuberculosis has been known to mankind since ancient times.

In ancient times, Consumption, Phthisis and The White plaque are the terms used to refer tuberculosis. The evidence of first tuberculosis infection happened about 9000 years ago. It spread to humans along trade routes and through domesticated animals. *Mycobacterium bovis* is disease causing organism that spread through animal domestication.

In the Neolithic era (9000 years ago), evidence of spinal tuberculosis is seen. Signs of the disease have also been found in Egyptian mummies dated between 3000 and 2400 B.C.

The first reference to tuberculosis in Non-European civilization is found in the *Vedas.*

Rigveda (1500 B.C) calls the disease ***YAKSHMA.*** The *Atharva veda* calls it ***BALASA.*** The ***SUSHRUTA SAMHITA***, written around 600 B.C, recommends that the disease be treated with breast milk, various meats, alcohol and rest. The ***YAJURVEDA*** advises sufferers to move to higher altitudes.

Hippocrates, in the book "Of the epidemics", describes the characteristics of the disease,

- Fever
- Colourless urine
- Cough with thick sputum
- Loss of thirst and appetite

They believed Phthisis to be hereditary but Aristotle disagreed and believed that the disease was a contagious.

In middle ages it was believed that royal touch of the Sovereign of England of France could cure disease due to the divine right of Sovereign. It is known as the **"KING'S EVIL".**

Girdamo Francastro is the first person to propose that phthisis was transmitted by an invisible virus. The incidence of tuberculosis grew progressively during the middle ages and Renaissance, displacing leprosy peaking between the 18^{th} and 19^{th} century.

In 1882, Prussian physician Robert Koch utilized a new staining method and applied it to the sputum of tuberculosis patients, revealing for the first time the causative agent of the disease, *Mycobacterium tuberculosis*.[3]

The first immunization against tuberculosis was developed from attenuated bovine strain tuberculosis by Albert Calmette and Camille Guerin in 1906. It was called "BCG" (Baccille Calmette- Guerin). The BCG vaccine was first used on humans in 1921 in France.

In 1944 Albert Schatz, Elizabeth Bugie and Selman Waksman isolated streptomycin from Streptomyces griseus. It was the first effective antibiotic against M. tuberculosis. In 1982, on the 100^{th} anniversary of Robert Koch's presentation the International Union Against Tuberculosis and Lung Disease (IUATLD) proposed that 24 March as official TB day.

Mycobacteria are slender rods, fungus like bacteria, aerobic, non-motile, non-capsulated, gram positive and acid fast bacilli.

Acid fast bacilli have waxy substance called mycolic acid in their cell walls which make them impermeable to many staining procedures. They are able to resist decolourization with acid alcohol. The stain used for acid fast bacilli is carbol fushsin by the Ziehl- Neelsen method or by fluorescent dyes.

Mode of transmission of this bacilli occurs through inhalation, ingestion, inoculation and transplacental route. Primary and secondary tuberculosis are the types of tuberculosis.[4]

Primary tuberculosis is the initial infection of the tubercle bacilli in a host. The bacilli engulfed by alveolar macrophages multiply and gives rise to a subpleural focus of tuberculous pneumonia, commonly located in the lower lobe or the lower part of the upper lobe (Ghon's focus). The hilar lymph nodes are involved.

The Ghon's focus together with the enlarged hilar lymph node constitutes the primary complex. This occurs about 3-8 weeks from the time of infection and is associated with the development of tuberculin hypersensitivity.

The secondary tuberculosis is due to reactivation of latent infection. It affects mainly the upper lobes of the lungs. The clinical features includes, referable to lungs,

- Productive cough may be with haemoptysis
- Pleural effusion
- Dyspnoea
- Orthopnoea etc,.

Systemic features,

- Fever
- Night sweats
- Fatigue
- Loss of weight and appetite

The diagnosis test includes,

- Positive Mantoux skin test
- Positive sputum for AFB
- Complete haemogram
- Chest x-ray
- Fine needle aspiration cytology of enlarged peripheral lymph node.[5]

Tuberculosis is the ninth leading cause of death worldwide. In 2017 it is estimated that1.3 million people were died from TB and 250,000 children died of TB in 2016.[6]

India is the country with highest burden of TB. The WHO TB statistics for India in 2017 give an estimated incidence figure of 2.79 million cases of TB. In this about 3% were found to be HIV positive. About 40% of the Indian population is infected with tuberculosis bacteria.

In Tamilnadu, Revised National Tuberculosis Control Programme (RNTCP) has reported for about 77,100,000 cases for TB.[7]

Tuberculosis treatment consists of a multi- drug regimen with duration of 6 to 8 months. The preferred first line drugs were,

- ✓ Isoniazid
- ✓ Rifampicin
- ✓ Pyrazinamide and
- ✓ Ethambutol

When the bacterial strains become resistant to one or more of these drugs, second line drugs are used. Second line drugs are,

- ✓ Streptomycin
- ✓ Kanamycin
- ✓ Fluoroquinolones
- ✓ Ethionamide and
- ✓ P- aminosalicylic acid

Drugs associated toxicity is an unfortunate outcome of this tubercular treatment. Hepatotoxicity is the major side effect for almost all first-line anti-tuberculosis drugs. Pyrazinamide accounts for exanthema and arthralgia.[8]

The emergence of MDR and XDR and complications of the anti-tuberculosis drugs there is an urgent need for alternative which could fulfil the need.

Attention across the world has focused towards traditional system of medicine, as it is affordable, safe, efficient and culturally acceptable.

Indian system of medicine is of great antiquity. Our country is blessed with a plethora of traditional medicines and practises. Siddha system of medicine, the foremost of all other medical systems, generated from Dravidian culture and it is flourished in the period of Indus valley civilization. It's origin dates back to BC 10,000 to BC 4000 (Sambasivampillai, 1931; Pillai, 1979) and it is exclusively practised in Tamilnadu.

Siddha denotes which means achievement in life arts such as,

- ➢ Alchemy (*Vaatham*)
- ➢ Medicine (*Vaithiyam*)
- ➢ Yoga (*Yogam*)
- ➢ Philosophy (*Thathuvam*)

Siddhars are spiritual scientists who explored and explained the reality of nature and its relationship to mankind. They were great philosophers and physicians and postulated the concept of spiritualism for self-improvement.

. There were many group of *Siddhars*, in which there are mainly 18 *Siddhars*, who are named as "*PATHINEN SIDDHARGAL"*.

"Medicine means the prevention of physical illness
Medicine means the prevention of mental illness
Prevention means to avert illness
Medicine therefore is the prevention of death"

This is an interesting definition of medicine by *Thirumoolar*. *Siddhars* used the *Siddha* medicine to retain their body and mind from ageing and also to prevent the body from diseases to attain spiritual perfection, a semi-divine state.

The *Siddhars* uniformly believed that a human body is composed of 96 basic factors. These are called as "*THATHUVAS"*. Traditional systems of medicine throughout the world are based on the humoral theory. There are three "HUMOURS" in *Siddha* system of medicine namely, *Vadham, Pitham* and *Kapham.* These are the combination of *Panchaboodhas.*

Earth and water form *Kapha*, fire *Pitha*, air and space form *Vadha.* These three *thadhus* are nourished by their respective elements present in food.

In *Siddha* system of medicine classification of diseases mainly based on *Vadha, Pitha* and *Kaph*a factors. The treatment in *Siddha* medicine is aimed at keeping the three *dhoshas* in equilibrium. So a proper diet, medicine and a regimen of life is advised for a healthy living and restore equilibrium of *dhoshas* in diseased conditions.[9]

In *Siddha, **"SAYAM"*** is the disease correlated with tuberculosis. It is also known as *"Ilaippu noi"* because of the main symptom of this disease are emaciation of whole body before the patient knows about his health. ***"EELAI"*** due to production of phlegm in large amount, ***"YAKSHMA"*** is because the disease is cross infected in many chronic diseases.

The major symptoms of this disease are evening rise of temperature, severe cough, increased sweat at night, emaciation of the body and the cause of the disease is starvation, any chronic diseases, malnutrition and immunosuppression.[10]

There are numerous single herb and poly herbal formulations are available in *Siddha* to treat tuberculosis. One of the polyherbal formulation mentioned for TB is ***"THIRIJADHATHI MATHIRAI"*** from the literature of *"**Anubhava Vaithiya Deva Ragasiyam**"* written by **Dr. K. Radhakrishnan L.I.M.**

"*Thirijadhathi mathirai" – **Thirisatham*** means *lavangapattai, lavangapathiri* and *elakkai.* It also contains *thippili, karkandu, dhratchai, adhimadhuram* and *karchurakai.*

In this *lavangapattai, lavangapathiri, elakkai* and *thippili* have anti-microbial activity against tuberculosis especially MDR- TB. *Elakkai* and *adhimadhuram* contain expectorant activity. They also posses immunomodulatory activity.

So the author considered the trial drug ***"THIRIJADHATHI MATHIRAI"*** will be effective, non-toxic, cost effective in treating TB. Though the medicine is present in literature there is no scientific validation to prove the efficacy and effectiveness of this drug. So an attempt was made to standardize this medicine through standardization protocol, toxicological profile, anti- tuberculosis, bronchodilator and anti- histamine activity in vitro.

2. AIM AND OBJECTIVES

AIM:

The present investigation was aimed to validate the safety and efficacy of the Siddha polyherbal formulation "*Thirijadhathi mathirai"* for its Anti tuberculosis In-Vitro and Bronchodilator and Anti histamine activity in animal model respectively.

OBJECTIVES:

The key objectives of the study are:

- Having a collective review of the literature.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug to physico-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment.
- Studying the toxicity profile of *Thirijadhathi mathirai* according to OECD guidelines.
- Determining the pharmacological activity (In Vitro anti-tuberculosis activity) of *Thirijadhathi mathirai*.
- Evaluation of bronchodilator activity of *Thirijadhathi mathirai* in guinea pigs.
- Evaluation of anti-histamine activity of *Thirijadhathi mathirai* by inducing bronchospasm in guinea pigs by exposing them to histamine aerosol under standardized conditions.
- Analyzing all the above study results to validate the benefits of *Thirijadhathi mathirai.*

3. REVIEW OF LITERATURE

3.1 DRUG REVIEW:

"THIRIJADHATHI MATHIRAI"

Ingredients:

- *Lavangapattai (Cinnamomum verum)*
- *Lavangapathiri (Cinnamomum tamala)*
- *Elakkai (Eletteria cardamomum)*
- *Thipilli (Piper longum)*
- *Karkandu (Sugar candy)*
- *Dried dhiratchai (Vitis vinifera)*
- *Adhimathuram (Glycyrrhiza glabra)*
- *Karchurakai (Phoenix dactilifera)*

3.1.1 GUNAPADAM ASPECT OF THE DRUG:

1. LAVANGAPATTAI:[11a]

Other Name:

Karuvapattai

Vernacular Names:

Eng : Bark of cinnamon

Tel : Lavangapatta

San : Twak

Mal : Lavangapatta

Kan : Dala-Chinni

Parts Used: Bark

Properties and Action:

Taste : Hot, sweet

Potency : Hot

Division : Sweet

Action : Carminative, Stimulant, Aphrodisiac

General Properties:

"தாதுநட்டம் பேதி சருவவிஷம் ஆசியநோய்
பூதகிர கஞ்சிலந்திப் பூச்சிவிடஞ் - சாதிவிடம்
ஆட்டுமிரைப் போடிருமல் ஆகியநோய்க் கூட்டமற
ஒட்டுமில வங்கத் துரி".

"சன்னலவங் கப்பட்டை தாங்குளிர்ச்சி யுண்டாக்கும்
இன்னுமிரத் தக்கடுப்பை யீர்க்குங்காண் - முன்னமுறும்
உந்திக் கடுப்பகற்றும் உண்மூலப் புண்போக்கும்
கந்தமிகு பூங்குழலே காண்."

\- அகத்தியர் குணபாடம்

Indications:

- It is useful to treat snake bite, spider bite, asthma and cough.
- It acts as refrigerant
- It cures internal piles and stomach discomfort.
- It is useful to treat diarrhoea

2. LAVANGAPATHIRI: [11b]

Other Names:

Thalisapathiri, Thamalapathiri

Vernacular Names:

Eng : Cassia cinnamon

Tel : Adavi- lavangapathiri

San : Tamalapathiram

Mal : Paccila

Kan : Kadu lavanga patte

Parts Used: Leaf

Properties and Action:

Taste : Hot

Potency : Heat

Division : Sweet

Action : Carminative, Stimulant, Stomachic, Diaphoretic

General Properties:

"மேகசுரம் சீதசுரம் வெட்டைசுவா சங்காசம்
தாகபித்தம் வாந்திசர் வாசியநோய் - மேகத்தின்
கட்டியொடு தாதுநட்டங் கைப்பருசி போக்கிவிடும்
இட்டஇல வங்கத் திலை."

- அகத்தியர் குணபாடம்

Indications:

- It is used to treat syphilitic fever, cough, thirst, asthma, vomitting and oral ulcer.

3. ELAKKAI: [11c]

Other Names:

Aanji, Korangam, Thudi

Vernacular Names:

Eng : Cardamom seeds

San : Ela

Mal : Elattari

Hin : Elachi

Parts Used: Seeds

Properties and Action:

Taste : Hot

Potency : Heat

Division : hot

Action : Carminative, Stimulant, Stomachic.

General Properties:

" தொண்டை வாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல்எழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்
பண்டை வெக்கை விதாகநோய் காசமும்
பாழுஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏல மருந்தே.....

- தேரன் குணபாடம்

Indications:

- It is used to treat cough, diarrhoea and thickened sputum.

4. THIPILLI: [11d]

Other Names :

Saram, Pippli, Kaman, Koli, Ambu, Arkathi, Kanai, Adhi marunthu, Vaidegi.

Vernacular names

English : Long pepper

Sanskrit : Pippali

Telugu : Pippilu

Kannada : Hippili

Malayalam : Thipilli

Part used: Dried fruit

Properties and action

Taste : Sweet

Nature : Hot

Division : Sweet

Action : Carminative, Stimulant.

General characters

"ஈளை யிருமலிரைப் புப்பசப் பிணிகள்
மாள வொழியாமல் வாட்டுமே - யாளுமுறை
பாங்காயறிந்து செய்வீர் பண்டிதத்தைப் பண்டிதரே
வேங்கை வாய்ப்பான் கணைமெய்"

- தேரன் வெண்பா

Indications:

It cures anaemia, fatigue, headache, sinusitis, pulmonary tuberculosis, cough and bronchial asthma.

Therapeutic uses:

Powder : Administered with honey will relieve cough, asthma and hoarseness.

Infusion : Infusion made of 10 peppers with honey makes a good expectorant

Oil : Rubbed externally in sciatica and paraplegia.

Fruit : Given to women after parturition for fever.

5. *KARKANDU* (sugar candy): [11e]

Other Names :

Punarpusam, Ekku, Vei

Vernacular names

English : Sugarcane

Sanskrit : *Ikshu*

Telugu : *Cheruku*

Kannada : *Khabbu*

Malayalam : *Karinpa*

Part used: Root, Sugar

Properties and actions:

Taste : Sweet

Nature : Cold

Division : Sweet

Actions : Demulcent, Antiseptic, Refrigerent, Laxative and Diuretic.

General characters:

" ஈறின் தடிப்பு மிருமலும்பல் வாந்திகளுஞ்
சீறுகப முட்டிணமுஞ் சேராதே - தேறியநற்
சொற்கண் டிளங்குயில்கள் சூழ மடவனமே
கற்கண் டெனவுரைக்குங் கால்."

- அகத்தியர் குணபாடம்

Indications:

- It is used to treat gum swelling, cough, vomiting and iyaazhal.

6. DRIED DHIRATCHAI: [11f]

Other Names:

Aravaaram, Kodimunthiri, Kodimunthirigai, Madhurasam, Kothirigai, Dhirakam, Palothamai.

Vernacular Names:

English : Grapes

Sanskri : Draksha

Telugu : Draksha

Kannada : Draksha

Part used: Fruit

Properties and actions:

Taste : Sweet

Nature : Cold

Division : Sweet

Actions : Laxative, Refrigerant, Diuretic, Nutritive.

Indications:

- It is used to treat constipation, anaemia and bleeding disorders.
- It can be given for the patients with fever, cough, jaundice and tuberculosis.
- It is used as refrigerant.
- It is used as tonic and improves the haemoglobin level.

7. ATHIMATHURAM: [11g]

Other Names:

Athingam, Mathugam, Kundri ver.

Vernacular names:

Sanskrit : *Yashti - madhukam*

English : Indian liquorice

Hindi : *Jathi - Madh*

Telugu : *Yashti - madhukam*

Malayalam : *Iratti - Madhuram*

Part used: Root

Properties:

Taste : Sweet

Nature : Cold

Division : Sweet

Actions : Demulcent, Laxative, Tonic, Mild Expectorant.

General characters:

"கத்தியரி முப்பிணியால் வருபுண் தாகங்
கண்ணோய்உன் மாதம்விக்கல் வலிவெண் குட்டம்
பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த
பித்தமத மூர்ச்சை விட பாகம் வெப்பந்
தத்திவரு வாதசோ ணிதங்கா மாலை
சருவவிடங் காமியநோய் தாது நட்டங்
குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து
குயப்புணும்போம் மதூகமெனக் கூறுங் காலே"

- தேரையர் குணபாடம்

Indications:

- It reduces excessive thirst, hiccough and leucoderma.
- It cures disease of bones, jaundice and burning micturition.
- Melts phlegm due to *Iyam*, and reduces *pitha kutrams*.
- It is used to treat faint, reduces over body heat.
- It induces the aphrodisiac activity in males.

Uses :[14b]

- The root is also used in irritable conditions.
- Root powder mixed with lime juice used as a remedy for swelling and pain.
- In China, the *Adhimathuram* root is a great important drug in Chinese pharmacy, for strengthening of bone and muscles and increasing physical strength and curing wounds.

8. ***KARCHURAKAI:***[11h]

Other Name: *Pereechu*

Vernacular names:

Sanskrit	:	*Kharjjuram*
English	:	Date palm
Hindi	:	*Kajur*
Telugu	:	*Karjuramu*
Malayalam	:	Perich- cha

Part used: Fruit

Properties:

Taste	:Sweet
Nature	:Heat
Division	:Hot
Action	:Demulcent, Laxative, Tonic, Nutritive, Diuretic.

General characters:

" வாயிலுண்டா மூற்றலை மாற்றும் பசியில்லை
யாயிலுண்டா லுண்டா மதுமேக - நோயுள்
பெருநீர் மறுக்குமினும் பெண்களைக் கூடில்
தருநீர்மை சூரக்காய் தான்."

- அகத்தியர் குணபாடம்

Indications:

- It is used to treat increased salivary secretion, diabetes and diarrhoea.
- It increases the appetite.

3.1.2 MODERN ASPECT OF THE DRUG:

1. LAVANGAPATTAI:

Botanical Name: *Cinnamomum verum*

Taxonomical Classification:[12]

Kingdom : Plantae

Subclass : Magnoliids

Order : Laurales

Family : Lauraceae

Genus : *Cinnamomum*

Species : *verum*

Fig.no.1 *Cinnamomum verum*

Distribution:[13]

It is native to Srilanka and found wild in Western and Southern India. It is cultivated in Malay Islands.

Parts Used: Bark

Botanical Description:

An evergreen tree, 8-16m high, with reddish brown bark having numerous small warts. Leaves ovate or elliptic ovate, thick, leathery, subacute or shortly acuminate, shining green on upper surface, main nerves 3-5 from petioles. Flowers minute, in axillary or sub-terminal cymes or panicles. Fruits dark, purple, single seeded berry.

Chemical Constituents:

Linalool, benzyl acetate, cinnamic aldehyde, eugenyl acetate, cinnamyl acetate, benzyl benzoate, cinncassiol C1 glucoside, cinncassiol C2 and C3, diterpenes, cinnamaldehyde, pinene, cymene, cumic aldehyde, esters of isobutyric acid, dipentenoids, cinnamyl alcohol, polymeric proanthocyanidin, arabinoxylan.

Actions and Uses:

The bark is aromatic, astringent, aphrodisiac, deodorant, stimulant, expectorant, febrifuge, diuretic and carminative. It is useful in bronchitis, asthma, cardiac diseases, diarrhoea, nausea and vomiting, urinary disorders, fever, halitosis and restoring the normal colour on the face. Cinnamon oil is stomachic, carminative, emmenagogue, styptic and useful in anorexia, inflammations, abdominal pain, toothache and tubercular ulcers.

Pharmacological Activities:

Anti-complement, anti-allergic, anti-bacterial, anti-microbial, anti-fungal, anti-oxidant, insecticidal and lipolytic.

2. LAVANGAPATHIRI:

Botanical Name: *Cinnamomum tamala*

Taxonomical Classification:[14]

Kingdom : Plantae

Subclass : Magnoliids

Order : Laurales

Family : Lauraceae

Genus : *Cinnamomum*

Species : *tamala*

Fig.no.2 *Cinnamomum tamala*

Distribution:[15]

A small evergreen, moderate sized tree growing in sub-Himalayan tracts from Jammu eastwards to Bhutan between altitude 900-2300 m and in Khasi hills, altitude 900- 1200m.

Macroscopic Characters:

Leaves are simple, linear lanceolate, 5 to 15 cm long, 3 to 6 cm broad, midrib prominent at the lower side, with three lateral veins arising from the base, converging towards the apex, margin entire, apex acute- acuminate, base symmetrical, upper surface smooth shining and leathery, lower surface slightly rough, somewhat pubescent, petiole 7 to 15 mm long, centrally grooved; dull green in colour. Odour pleasant and aromatic, taste sweet, spicy and mucilaginous.

Microscopic Characters:

PETIOLE: TS of the petiole is oval in outline with a slight central depression on the upper side, epidermis covered with thick cuticle and bears trichomes like that of leaf. Hypodermis consists of 4 to 8 rows of collenchymatous tissue, encircling the wide parenchymatous cortex, filled with starch grains and oil globules, more crowded around the centrally located twin, unequal sized fibro-vascular bundles. Cells containing volatile oil and mucilage canals transversed throughout the cortex.

LEAF: TS passing through the midrib convexly protrudes on the lower side and obliquely on the upper side and shows centrally located twin fibro- vascular bundles of unequal size, unlike the lateral vein where lies only one meristele. Midrib contains collenchymatous tissue above and below. Meristele consists of radially arranged central xylem and phloem, encircled by a ring of pericyclic fibres. Two rows of palisade lie underneath the upper epidermis. Glandular trichomes with unicellular stalk and unicellular head, sessile trichomes, pearl gland are also present, oil containing cells and circular to oval mucilage canals transversed throughout the lamina and midrib tissue and starch grains in mesophyll cells.

Chemical Constituents:

Eugenol, linalool, ∞- pinene, camphene, β- pinene, benzaldehyde, myrcene, limonene, p- cymene, benzyl acetate, ∞- terpineol, cinnamaldehyde, geraniol, linalool acetate, benzyl cinnamate and tannins.

Actions and Uses:

The dried leaves are useful to treat cough, asthma and bronchitis.

Pharmacological actions:

Hypoglycaemic, hypolipidaemic, anti-bacterial activities, fungicidal, fungistatic.

3. ELAKKAI:

Botanical Name: *Elettaria cardamomum*

Taxonomical Classification:[14]

Kingdom : Plantae

Subclass : Monocots

Order : Zingiberales

Family : Zingiberaceae

Genus : *Elettaria*

Species : *cardamomum*

Fig.no.3 *Elettaria cardamomum*

Distribution:[16]

It is a native of the moist evergreen forests of South India, growing wild in the Western Ghats, between 800- 1600 m. It is commonly cultivated in Kerala, Karnataka and Tamilnadu.

Parts Used: Seed

Botanical Description:

A tall herbaceous perennial with branching subterranean root stock and several erect stems up to 3m high. Leaves 30-90 cm long, subsessile, elliptic or lanceolate with sheathing base.

Flowers borne in panicles, 60-120 cm long, arising from the bse of vegetative shoots; panicles generally upright at first and ultimately pendent or lying on the ground; flowers white or pale green with a central lip streaked with violet.

Fruits trilocular, subglobose or fusiform to ovoid capsule. Seeds 15-20 per pod, brownish black, angled, rugose, covered with a thin mucilaginous membrane.

Chemical Constituents:

∞- Pinene, sabinene, myrcene, limonene, cineol, cymene, methyl heptenone, linalool, linalyl acetate, ∞ & β- terpineol, ∞- terpinyl acetate, borneol, nerylacetate, geraniol, nerol, neolidol, heptacosane, 1, 8- cineole, camphene, terpinene, ∞- humulene, E-4, 8- dimethyl- 1, 3, 7, 11- tridecatetraene (essential oil of fruits and leaves).

Actions and Uses:

Seeds are aromatic, refrigerant, stimulant, carminative, digestive, stomachic, diuretic, cardiotonic, alexiteric, expectorant, tonic and abortifacient. They are useful in anorexia, vomiting, giddiness, dyspepsia, gastric disorders, asthma, bronchitis, haemorrhoids, strangury, dysuria, renal and vesical calculi, halitosis, cardiac disorders, hyperdipsia, burning sensation, debility and defects of vision.

Pharmacological Activities:

Hepatoprotective, anti- inflammatory, analgesic, antispasmodic, antimicrobial and antifungal.

4. THIPPILI:

Botanical Name: *Piper longum*

Taxonomical Classification:[17]

Kingdom : Plantae

Subclass : Magnoliids

Order : Piperales

Family : Piperaceae

Genus : *Piper*

Species : *longum*

Fig.no.4 *Piper longum*

Distribution:[18]

It occurs in hotter parts of India from Central Himalayas to Assam, Khasi and Mikir hills, lower hills of Bengal and evergreen forests of Western Ghats from Konkan to Travancore.

Parts Used: Fruit, root

Botanical Description:

A slender, aromatic climber with perennial woody roots, stems creeping, jointed. Leaves ovate, cordate, subacute, entire, glabrous. Spikes cylindrical, pedenculate, male larger and slender, female 1.3- 2.5 cm long. Fruits ovoid, yellowish orange, sunk in fleshy spike.

Chemical Constituents:

Stem and roots: Two alkaloids piperlongumine and piperlonguminine characterised as N- (3, 4, 5- trimethoxy cinnamoyl)- Δ- piperidin- 2- one and isobutylamide of piperic acid respectively.

Essential oil from the dried fruit: n- hexadecane, n- heptadecane, n - octadecane, n - nonadecane, n- eicosane, n-heneicosane, ∞ - thujene, terpinolene, zingiberene, p - cymene, p - methoxy acetophenone, traces of dihydrocarviol, phenylethyl alcohol and two sesquiterpenes.

Roots: Piperine, piplartine, triacontane, dihydro- stigmasterol, reducing sugars, glycosides, sesamin and methyl - 3, 4, 5 - trimethoxycinnamate.

Stem and fruits: Major alkaloid piperine and sesamin

Essential oil: Sesquiterpene hydrocarbon, caryophyllene, a sesquiterpene alcohol, carbonyl compound.

Fruit: N- isobutyldeca- trans- 2- trans- 4 - dienamide, piperine, piplartine and a lignin d- sesamin, two piperidine alkaloids- pipernonaline and piperundecalidine.

Seed: Sylvatin, sesamin and diaeudesmin.

Actions and Uses:

The root is bitter, thermogenic, tonic, diuretic, purgative, expectorant, anthelmintic, stomachic, digestive and emmenagogue. They are useful in gout, limbago, dyspepsia, apoplexy, gastralgia and splenopathy.

Dried spikes are acrid, vermifuge, mildly thermogenic, stomachic, aphrodisiac, carminative, expectorant, febrifuge, laxative, digestive, emollient and antiseptic. They are useful in anorexia, dyspepsia, vomiting, flatulent colic, diarrhoea, cholera, dysentery, asthma, bronchitis, coryza, hiccough, consumption, gastric disorders, epilepsy, insomnia, fever, gonorrhoea, haemorrhoids, gout and lumbago.

The fruits are used after child birth to check postpartum haemorrhage, as a cholagague in bile duct and gallbladder obstruction.

Unripe fruit is used as a tonic. A decoction of immature fruits and roots is used in chronic bronchitis, cough and cold; also used in palsy, gout, rheumatism and lumbago.

Pharmacological Activities:

Antibacterial, anti-inflammatory, insecticidal, antimalarial, CNS stimulant, anti- tubercular, anti- helminthic, hypoglycaemic, antispasmodic, cough suppressor, anti-giardial, immunostimulatory, hepatoprotective, analeptic, antinarcotic and antiulcerogenic.

5. KARKANDU:

BOTANICAL NAME: *Saccharum officinarum*

TAXONOMICAL CLASSIFICATION:[14]

Kingdom : Plantae

Subclass : Monocots

Order : Poales

Family : Poaceae

Genus : *Saccharum*

Species : *officinarum*

Fig. no.5 *Saccharum officinarum*

Distribution: [19]

It is extensively cultivated in hotter parts throughout India.

Parts Used: Root, stem

Botanical Description:

Perennial tall herb, upto 6.5 m high with stems of varying thickness and colour, many noded. Leaves 1.6 m x 6 cm, linear lanceolate, midrib very stout, erect or drooping. Raceme upto 10 cm long, fragile, pedicel short. Spikelets lanceolate, upto 0.1 cm long, usually surrounded by long silky hair from their base. Grain oblong to subglobose, subterete, flesh coloured.

Chemical Constituents:

5-0- Methylapigenin and 3',4',5,7-tetrahydroxy-3-6-dimethoxyflavone, aminoacids, phenylalanine, arginine, saccharetin, methionine, pantothenic acid, biotin.

Action And Uses:

They act as Expectorant, Haemostatic and Tonic. They are useful in cough, bronchitis, anaemia, emaciation, general debility.

Pharmacological Activities:

Antioxidant, Expectorant and Tonic.

6. DHIRATCHAI:

Botanical Name: *Vitis vinifera*

Taxonomical Classification:[14]

Kingdom : Plantae

Subclass : Rosids

Order : Vitales

Family : Vitaceae

Genus : *Vitis*

Species : *vinifera*

Fig.no.6 *Vitis vinifera*

Distribution:[20]

It is cultivated in Jammu-Kashmir, Himachal Pradesh, Uttar Pradesh, Rajasthan, Punjab, Haryana, Delhi, Maharashtra, Karnataka, Andhra Pradesh and Tamilnadu.

Parts Used: Ripe fruit (dried), leaf, flower, stem.

Botanical Description:

A large, perennial tendril climber, tendril leaf opposed, often bifid. Leaves simple, rotund-cordate or orbicular cordate, dentate, 3-7 lobed, 10-12 cm across, glabrous above, tomentose beneath. Flowers in long peduncled, leaf-opposed cymes, greenish or white. Fruits (berry) globose, ovoid or oblong, varying in size, pale green or purple. Seede 2-4, oblong-obovoid, brown, with a discoidal tubercle on the back.

Chemical Constituents:

Fruit: 3-monoglucosides of delphinidin, petunidin, peonidin, malvidin, acetyl and coumaryl glucosides, biflavanoids, malic acid, tannic acid, dehydroascorbic acid, β-sitosterol, ergosterol, glucose, fructose, galactose, mannose, arabinose, rhamnose and aminoacids like alanine, arginine and proline.

Action and Uses:

The fruits are sweet, refrigerant, laxative, demulcent, cardiotonic, haematinic, diuretic, aphrodisiac, rejuvenating, nervine tonic, digestive, suppurative, expectorant and tonic.

They are useful in burning sensation, constipation, amentia, cardiac debility, haemoptysis, haemorrhages, anaemia, consumption and wasting diseases, cough, asthma and bronchitis.

Pharmacological Activities:

Antifungal, angiotensin-converting enzyme (ACE) activity, hepatoprotective, antioxidant, wound healing, antibacterial, breast cancer suppressor, antimutagenic and cardioprotective.

7. ADHIMADHURAM:[21]

Botanical Name: *Glycyrrhiza glabra*

Taxonomical Classification:

Kingdom : Plantae

Subclass : Rosids

Order : Fabales

Family : Fabaceae

Genus : *Glycyrrhiza*

Species : *glabra*

Fig.no. 7 *Glycyrrhiza glabra*

Distribution:[22]

It is distributed in the Sub-tropical and warm temperature regions of the world, chiefly in the Mediterranean countries, South Europe, Asia Minor, Egypt, Turkistan, Iran, Siberia, Persia, Arab countries and Afganistan.

In India, it is reported to be cultivated in Baramulla, Srinagar, Jammu, Dehra Dun, Delhi and South India.

Parts Used: Root

Botanical Description:

It is hardy herb or undershrub attaining a height up to 2 m; leaves multifoliate, imparipinnate; flowers in axillary spikes, papilionaceous, lavender to violet in colour; pods compressed containing reniform seeds.

Chemical Constituents:

Glycyrrhizine, prenylated biaurone, 4-methyl coumarin, liqcoumarin, isoflavone, quercetin,

Action and Uses:

The roots are sweet, refrigerant, emetic in large doses, tonic, diuretic, demulcent, mild laxative, aphrodisiac, expectorant, emmenagogue, haemostatic and intellect promoting.

They are useful in hyperdipsia, cough, bronchitis, ulceration of urinary tract, retention of urine, gastralgia, gastric ulcer, cephalgia, fever, skin diseases, ophthalmic diseases, pharyngitis, consumption, epilepsy, anaemia, urticaria.

Decoction of root is good wash for falling and greying of hair. It is externally applied for cuts and wounds.

Pharmacological Activities:

Smooth muscle depressant, antimicrobial, hypolipidaemic, antiviral, hypotensive, hepatoprotective, antiexudative, expectorant, antimutagenic, anti-inflammatory.

8. *KARCHURAKAI:*

Botanical Name: *Phoenix dactylifera*

Taxonomical Classification:[14]

Kingdom : Plantae

Subclass : Monocots

Order : Arecales

Family : Arecaceae

Genus : *Phoenix*

Species : *dactylifera*

Fig.no.8 *Phoenix dactylifera*

Distribution:[23]

Dates have been a staple food of the Middle East and the Indus Valley for thousands of years. Dates cultivated in eastern Arabia, Iraq, Mesopotamia.

Parts Used: Fruit

Botanical Description:

Date trees typically reach about 21–23 metres (69–75 ft) in height,[7] growing singly or forming a clump with several stems from a single root system.

The leaves are 4–6 metres (13–20 ft) long, with spines on the petiole, and pinnate, with about 150 leaflets.

The leaflets are 30 cm (12 in) long and 2 cm (0.79 in) wide. The full span of the crown ranges from 6–10 m (20–33 ft).

The date palm is dioecious, having separate male and female plants. They can be easily grown from seed, but only 50% of seedlings will be female and hence fruit bearing and dates from seedling plants are often smaller and of poorer quality.

Most commercial plantations thus use cuttings of heavily cropping cultivars. Plants grown from cuttings will fruit 2–3 years earlier than seedling plants.

Chemical Constituents:[24]

Anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids.

Actions and Uses:

It is used to treat loss of appetite. It also enhances the immunity and used as tonic.

Pharmacological Activities:[25]

Free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities.

3.2.1 SIDDHA ASPECT OF THE DISEASE

Tuberculosis *(Ilaippu noi)*

Introduction:

Tuberculosis is one of the major diseases which occur due to the default of *Kapha*. This disease is also called *Ellai, Kaba noi,* King of diseases *(arasa noy), Kshayam, Rajayakshma,* Y*akshma and Shushashai*. As the body gets emaciated much in this disease, it is called emaciating disease or *Ilaippu noi* in *Tamil.*

Aetiology:

- Frequent starvation
- Wakefulness at night without sleep
- Excessive hard work beyond one's ability
- Suffering from many other diseases for a longer duration
- Suffering due to poverty
- Mental depression due to imprisonment
- Consuming poor nutritious food
- Living in places of poor ventilation
- Using cannabis
- Excess intake of pungent and salty food

- Syncope to a large extent
- Consuming stagnant water
- Consuming tasteless foods
- Consuming water on limestone bed
- Wandering in hot sun
- Being near to excess heat
- Excess sorrowfulness
- Telling lies
- Stealing
- Being false to relatives
- Speaking ill of others
- Speaking unparliamentary words

These are the reasons for tuberculosis according to Siddha literatures. In general, the basic factors in the incidence of this disease are the debilitating condition of the body with loss of immunity and mental disturbances. This will also occur as an associated disease of diabetes mellitus, etc.

Signs and symptoms

- Rise of temperature in the evening
- Sore throat
- Rhinitis
- Loss of brightness of vision
- Cough
- Accumulation of sputum
- Pleurisy
- Deafness
- Vomiting
- Excess diarrhea
- Sweating at night
- Anorexia
- Craving
- Loss of appetite
- Being always dull

An illusory feeling of mud, stones, dust and ants being mixed with food substances, trembling due to a false feeling of paleness of the body, oedematous legs and severe emaciation which occur after a prolonged illness.

Feeling of sadness on seeing one's own body repeatedly.

Hallucinations like burning of towns, dryness of ponds, rivers and lakes, attack by dangerous birds, snakes etc... and destruction of forests and mountains, Overgrowth of nails and hairs.

நோய்த் தொகுப்பு வரலாறு:[26]

"தானானவிளைப் பெனுஞ்சயம் பனிரெண்டாகும்
தாக்கான பிரமமொன்று சயந்தா னென்று
வானான ராஜட்சம் வைசியச் சயந்தான்
மருவுசூத்திர சயந்தான் வாதச் சயந்தான்
பூனான பித்தச்சயம் சேட்பத் தோடு
பிரண்டதோர் வாதபித்தச் சயமு மாகும்
கானான வாதசேச் டுமட்ச யந்தான்
கரும்பித்தச் சேட்டுமத்தின் சயமு மாமே.

ஆகின்ற விகாரச்சயந் தொந்தச் சயந்தான்
அடங்கலுமே பனிரெண்டு விதமு மாகும்
வேகின்ற கஞ்சாவால் மிகுந்த வுப்பால்
மீறியதோர் காரத்தால் மிகம யக்கால்
தோகின்ற மருந்தீட்டாற் சுனை நீரால்
ருசியில்லாப் பதார்த்தங்கள் சுக்கான் தண்ணீர்
வாகின்ற வக்கினியால் மிகுந்த வெய்யில்
வருத்தத்தில் சயரோக மருவுங் காணே.

காணவே கற்பழித்தல் பொய்யே சொல்லல்
களவாடல் பிறர்பொருளைக் கண்டிச் சித்தல்
ஆனவே யடுத்தோடரைக் கெடநினைத்தல்
அகதிபர தேசியரைக் யகந்தை சொல்லல்
ஊனவே பந்துவை வஞ்சித்தே யுண்ணல்
உதாசினமா இகழ்ந்தோரை இகழ்ச்சி பேசல்
வேணவே காதினிற் கேளாத சொல்லை
மிகச்சொன்னோர் சயத்தில்வந்து மெலிகு வாரே."

- யூகி முனிவர்

Classification of the disease

Tuberculosis has the characteristic features of destroying all the seven body tissues and reducing the strength and stamina of the body. This disease is classified not only on the basis of the affected life forces but also of the *Vayu's* like *Uthanan and Abanan*. The deranged life forces and *Vayu's* specifically attack certain organs or places and so the signs and symptoms of the diseases vary according to the site of the lesion. On this basis, *Yugi* classified the disease into 12 types in Siddha system; *Yugi's* classification alone is generally followed.

It is as follows:

1. *Brahma ilaippu noi*
2. *Rasa ilaippu noi*
3. *Vaishya ilaippu noi*
4. *Sutthira ilaippu noi*
5. *Vatha ilaippu noi*
6. *Pitha ilaippu noi*
7. *Kapha ilaippu noi*
8. *Vatha pitha ilaippu noi*
9. *Vatha Kapha ilaippu noi*
10. *Pitha Kapha ilaippu noi*
11. *Vihara ilaippu noi*
12. *Thontha ilaippu noi*

Signs and symptoms of different varieties of tuberculosis

1. Brahma ilaippu noi

Emaciation, cough, expectoration of sputum along with watery discharge from lesions, blood-stained sputum, reduction of body temperature, bleeding from eyes, fever, persistent mental confusion, loss of taste and vomiting.

2. Rasa ilaippu noi

Head ache, pain in the chest, expulsion of yellowish sputum, pain in the trachea, change in voice, pain all over the body, heat throughout the body and vomiting with continuation of cough are the signs and symptoms of this disease.

3. Vaishya ilaippu noi

Weakness in both the limbs, inability to tolerate the sunlight, fever with excess perspiration, cough with expectoration, anaemia, constipation, intense suffering, yawning and shivering of the body are the signs and symptoms of this disease.

4. Sutthira ilaippu noi

Inflammation of the eyes, lacrimation in the eyes, cough, ulceration of trachea, shivering due to cold in the early morning, syncope, pain, sweating, vomiting and sputum mixed with vomitus, stickiness of sputum in throat, burning sensation, piercing pain, yawning and constipation are the signs and symptoms of this disease.

5. Vatha ilaippu noi

Emaciation of the body, headache, hiccough, pain in the ribs, pain in the centre of the back, vomiting, fever, thirst, pain in the shoulders, hands, legs and neck, diarrhoea, cough, loss of sleep, bitterness in the tongue and chest pain (piercing pain) are the signs and symptoms of this disease.

6. Pitha ilaippu noi

Dyspnoea, shrinking in the sides of the chest and neck, cough with expectoration, haematemesis, sore throat, syncope, indigestion, anaemia, jaundice, yellow discolouration of urine, fever, shivering with cold, sweating, excess coldness, muscle pain, intense suffering and discomfort are the signs and symptoms of this disease.

7. Kapha ilaippu noi

Vomiting in continuation of cough, sputum in vomitus, belching or eructation, loss of taste, diarrhoea, sore throat, pallor of the body, fatigue, rhinitis (running nose), flatulence (stony dullness may also be seen), burning sensation in the body, indigestion and intense suffering with discomfort are the signs and symptoms of this disease.

8. Vatha Pitha ilaippu noi

Pain all over the body, piercing pain, fever, thirst, expectoration, tasteless, cough, excess flatus, burning sensation in the body, syncope, ptyalism, scanty micturition, black discolouration of the body, excess coldness, dyspnoea, sneezing, piercing pain in stomach, lacrimation, sour taste and dryness of the chest are the signs and symptoms of this disease.

9. Vatha Kapha ilaippu noi

Sore throat, hoarseness of voice, fever, cough, pain all over the body, expectoration, weakness of the body, loss of appetite, anorexia, pallor of the body, atrophy of the limbs, palpitation in pulse, itching in the lower end of the bridge of the nose are the signs and symptoms seen in this disease.

10. Pittha Kapha ilaippu noi

Excess sleep, excess laziness, fever, vomiting, sputum in vomitus, diarrhoea, cough and haemoptysis, odourful sputum, giddiness, depressive psychosis, thirst, sinusitis, shivering with cold, sputum accumulation in chest and dryness of nose are the signs and symptoms of this disease.

11. Vihara ilaippu noi

Hallucination of sounds in the ear, pallor conjunctiva in both eyes, losing of stamina and strength of the body, emaciation of the body, ulceration, discharge, syncope, pain in the sides of the chest, haemoptysis, foul smell in the mouth and breath, excess appetite, burning sensation in the body, pain in the body, chillness in the extremities, irritation and congestion in uvula are the signs and symptoms of this disease.

12. Thontha ilaippu noi

Change of voice, fever, cough with haemoptysis, bad odour in mouth, shivering, diarrhoea, syncope, blackish discolouration of tongue, aloe's smell on the body, talkativeness, anuria and constipation, vomiting, dyspnoea, pungent irritation in throat, cough and bitterness of mouth are the signs and symptoms of this disease.

Curable varieties of the disease:

Brahma *ilaippu noi, Rasa ilaippu noi, Vaishya ilaippu noi*, *Pitha ilaippu noi*, and *Kapha ilaippu noi* are curable by medicine.

Incurable varieties of the disease

Sutthira ilaippu noi, Vatha ilaippu noi, Vatha Kapha ilaippu noi, *Pitha Kapha ilaippu noi, Vihara ilaippu noi, Thontha ilaippu noi* and *Vatha pitha ilaippu noi* are incurable by treatment.

The above said classifications are a generalized one as stated by *Yugi*. Another type of classification is based on the organs in which the deranged life forces and *Vayu's* are responsible for the occurrence of TB. It is as follows:

Tuberculosis which occurs in the organs below the umbilicus

The tuberculosis occurs in the intestine just below the umbilicus occurs intestine and its allied organs. The signs and symptoms of this disease will be as that of bronchial tuberculosis. In addition to those, frequent loose watery motion will be present. Body will be very much emaciated. Distension of lower abdomen, atrophied extremities and shrunken face will be seen. Fever will be frequent. Many nodules will be seen below the skin of distended abdomen. Day by day, the distension of the abdomen will increase and diarrhoea also occurs. Pain in the abdomen and accumulation of water in peritoneal cavity as that of ascites will be present.

Tuberculosis in the organs below the chest

This disease occurs in liver and kidney. The liver enlarges day by day. In the same manner, kidney also swells. Frequent micturition and incontinence of urine will be present. As in the other types of tuberculosis, here also the body will be emaciated. There will be unbearable pain in the lower back and in the hip.

Tuberculosis pertaining to the skin

Mostly, this disease occurs in the young age or middle age group. Erythema nodosum will be seen in nose, jaws, toes and in hands. It will become swollen and ulcerated. Alternative granulation and ulceration will occur. Ulcer may become permanent without getting healed.

Tuberculosis in nasal cavity

Frequent obstruction in nasal cavity and sneezing, cough and rhinitis will be present. The defect which occurs in nose will persist for a longer duration. However, watery discharge will become hard as that of sputum. Sometimes, it may fall from the nose as a hard mass. In one or both sides of the nasal bridge, there will be ulceration with some swelling. This ulcer will not be reddish as other ulcers. Apart from this, the general signs and symptoms of the disease will continue one after the other. This disease occurs rarely.

Tuberculosis in throat

As the sputum increases in pharynx and larynx, the tone of the voice is reduced. This is not easily curable. As it continues for a longer period, emaciation of the body occurs and other symptoms of tuberculosis will continue one after the other. On both sides of the pharynx, there will be swellings covered with whitish membranes. Ulceration and pitting will also be seen in the mass. Hoarseness of voice will persist for a longer duration, and it is not easily curable by any medicines.

Tuberculosis in neck

Around the neck, the lymph glands will be enlarged and so the glandular swellings will be seen around the neck as garland. They will be painful and the swellings will increase in size day by day. The swellings in the neck appear first with pain, then they enlarge and join with one another and look like a garland in the neck. Mostly, this disease occurs in children.

Day by day, the glandular swellings will increase to a bigger round, hardened mass round the neck. Sometimes, they ripen and open. This ulcer will not heal easily. It makes the individual emaciated. Fever, cough and diarrhoea will continue, and finally death will occur.

Tuberculosis of the chest

This disease mainly affects the lungs and spreads slowly. Body will get emaciated without the knowledge of the patient. Anorexia will be present. Running nose, mild cough with expectoration and sputum being lesser but sometimes stained with blood will manifest.

Hoarseness of voice with sore throat, rise of temperature in the evening and obstruction in breathing will be seen. Pleurisy and hyper pyrexia will get associated with this. Sometimes, there will be severe fever; cough and emaciation will be present. Sometimes, intermittent fever will be present for a longer duration and then severe cough and expectoration to a large extent will continue. The fever will be present throughout the day. For some individuals, without showing any of the above symptoms, the strength and stamina of the body alone will be reduced day by day. Afterwards, cough occurs slowly and then increases.

For some people, *Kapha* fever occurs at first, and it will get decreased. Afterwards, fever may continue with emaciation of the body. Thus occasion of tuberculosis ocurrs. The other symptoms will follow one after the other afterwards.

Tuberculosis in the axillary region:

This disease occurs after the age of 10-15 years. Along with the above said symptoms, glandular swelling will be seen in axilla.

Tuberculosis of the joints

Mostly, it occurs in knees and elbow joints of children. The swelling will resemble the inflammation of a joint. One or more joints will be affected. The swelling in joints will increase slowly little by little. The swelling is due to accumulation of fluid in the joints. Along with this, mild fever and cachexia will be seen.

The disease may also affect the hip joint (ball and socket joint) in which the upper end of the femur and ileum join together. In this disease, mostly the upper end of the femur will be affected. The bone in that area is putrefied and becomes shortened. Because of this, the leg in the affected side will be shorter than the unaffected leg. There will be difficulty in adduction of the affected leg. The body will be emaciated and also the other symptoms of tuberculosis will persist.

Tuberculosis of the bone

The bones in the vertebral column from top to bottom may be affected by this disease. Signs and symptoms will vary according to the affected bone.

When the disease occurs in the lower part of the vertebral column, degeneration of the bone occurs in any of the sides of the bone and so the spinal cord may be bent because of the strength of the bone being lost due to degeneration. Though there is no pain at the lesion, severe pain will occur while there are tapping and beating on the head. Pain will also be felt while bending. In the diseased condition of lumbar vertebrae, there will be shortening of leg.

When the disease affects the bone above that level, there will be pain in the buttocks and lower abdomen. When the disease affects the thoracic vertebral bones, there is pain in the stomach, and it also causes inability of the spine to bend forward, backward or sideways. The lateral curvature is called scoliosis. In potts disease or tuberculosis of the spine, excessive backward curvature of the dorsal spine occurs. This is called kyphosis. Kypho scoliosis or hibbus occurs in the thoracic spine. When cervical spine gets affected, head is erected and the shoulders support the head and so there is inability to move the head independently but its movement is possible along with the body.

In tuberculosis, sometimes the affected bone gets putrefied and pus may form. The pus moves through thin membranes and gets accumulated in various places according to the sight of the affected bone. It may be the lower part of the mouth, sides of the chest, or in the groins of the thigh. It will appear like abscess and may open.pus will come out from the opening.

If nerves get affected or compressed due to degenerations or putrefication of bones, the function of the nerves gets affected and so the organs to which the nerves supply also go dysfunctional. The supplying area of the nerves will have gnawing pain, piercing pain and numbness. Sometimes, paraplegia may occur. Micturition will be difficult (inability to pass urine). Death may occur if the cervical bones get affected.

Treatment of Tuberculosis:

This disease occurs due to increase of *Kapha* and due to reduction in the strength of the seven body tissues (Udal kattukal). [(34)] In this condition, the *Vayu* in the body get stimulated. The stimulated *Vayu* cause the disease in their site of existence. So the main aim of the treatment of this disease should be to normalize the

increased life force and the other life forces which join to it. The other aim should be to increase the strength and stamina of the tissues debilitated in this disease. Medicine should be given to set right the affected *Vayu* also. As in this disease loss of appetite is also an important symptom, medicine should be given to stimulate appetite also. Rich diet and medicine should be given to improve the stamina of the body. Vomiting and diarrhoea may be induced to normalize the conditions of the life forces. Vomiting is needed to expel the accumulated sputum in lungs and to reduce the increased *Kapha*. For inducing vomiting, milk, ghee, oil of emetic nut (*Randia dermetorum*) and decoction of emetic nut must be given. In the same manner, oil of Tirunelveli senna (*Cassia anciolata*) may be given for inducing diarrhoea. Diet and medicines to stimulate appetite and to strengthen the body may be given. By these methods, the altered life forces will come to normal. In continuation of this, vital and powerful medicines like calcinated white and red powders of mercury, gold, etc., which act directly against the disease must be given.

3.2.2 MODERN ASPECT:

Tuberculosis still remains one of the world's most prevalent infectious diseases. We still have an incomplete knowledge to understand the nature of the virulence of the tubercle bacillus and the nature of the host response to the tuberculous infection. Neither do we have a very clear understanding of the reason for the rise and fall in the number of cases of tuberculosis, nor is there a general appreciation of the inadequacies of the public health control methods that have been used to combat the disease.

The Mycobacterial World

Scientific classification:

Kingdom : Bacteria

Class : Actinobacteria

Order : Actinomycetales

Suborder : Corynebacterineae

Family : Mycobacteriaceae

Genus : *Mycobacterium*

Species : *tuberculosis*

Tubercle bacilli are non-motile, non sporing non capsulate straight or curved rods about 3 * 0.3 mm in size. [27]

Mycobacterium tuberculosis is an obligate aerobe. For this reason, in the classic case of tuberculosis, MTB complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, and a physiological characteristic that may contribute to its virulence.

Cell Wall Structure

The cell wall complex contains peptidoglycon, but otherwise it is composed of complex lipids. Over 60% of the Mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components, mycolic acids, cord factor and wax-D. [28]

Mycolic acids are unique alpha-branched lipids found in cell walls of Mycobacterium and Corynebacterium. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic Acids are thought to be a significant determinant of virulence in MTB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.

Cord Factor is responsible for the serpentine cording mentioned above. Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of MTB.

Wax-D in the cell envelope is the major component of Freund's complete adjuvant (CFA).

The high concentration of lipids in the cell wall of *Mycobacterium tuberculosis* has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- Resistance to lethal oxidations and survival inside of macrophages

Metabolism of mycobacteria

- *Mycobacterium tuberculosis* requires iron for essential metabolic pathways. Because iron is not freely available in the host, pathogens must actively compete for this metal to establish an infection but they must also carefully control iron acquisition as excess free iron can be extremely toxic.[29]
- The fact that iron has been known to be the key player required for its survival and ability to spread infection, the organism must carefully balance iron acquisition with iron uptake for its infectivity. Conversely, this iron homeostatic process could be disrupted to interfere with the survival and replication of this bacterium in host.[30]
- In mycobacteria the protein regulates the tricarboxylic acid cycle and glutamate synthesis by direct binding and regulation of three enzymes that use α-ketoglutarate.[31]
- The sulfate assimilation pathway of *Mycobacterium tuberculosis* is responsible for the biosynthesis of sulfur-containing metabolites that influence bacterial pathogenesis.[32]

The number of problems presented by the organism of tuberculosis.

These problems may be discussed under four headings.

1. Variability 2. Virulence 3. Biochemistry and 4.Types.

Tissue changes in tuberculosis including formation and healing of cavity changes in the tissues caused by the tubercle bacillus need not be characteristic. A simple abscess or an area of infiltration with lymphocytes and plasma cells may be tuberculous in origin, especially in mucous membrane (for example, in the larynx) or in tubercular organs such as the kidney or epididymis. Usually, however, the alterations in the tissues have certain features which may be taken as typical characters of tuberculosis. These may be grouped into early and later changes.[33]

1) Tuberculous tissue reactions may be grouped into early and late reactions.
2) The early response to the invasion of tubercle bacilli consists of tissue alteration (necrosis), exudation and local cell proliferation. The last-named leads to infiltration with macrophages and after the acute irritation have subsided and after then the formation of epithelioid cells, giant cells and tubercles occurs. The tuberculous giant cells (of the langhans type) are, not infrequently, endothelial and vascular offshoots, i.e. abortive vessels.
3) These early reactions do not occur in any particular order. One type of reaction may be present without traces of the others. From the very first local cell proliferation may occur and there is no need to postulate invisible initial stages of cell necrosis or leucocytic exudation.
4) All types of early reaction may occur in any affected organ or part of organ (e.g. interstitial or parenchymatous tissue)
5) Predominate, are the type, number, virulence and chemical constitution of the bacillus. Other factors are the varied susceptibility of different animals and of different organs and tissues to tuberculous infection.
6) Generally, in the causation of the tubercular response,
    a) The action of the bacillus as a fine particulate body,
    b) Allergy, and
    c) Specific action of certain compounds of the bacillus must be considered. Cell proliferation characteristic of tuberculosis was said to be elicited by the phosphatides of the bacillus. The application of this to human and experimental conditions, however, meets with difficulties.
7) The later tissue reactions to the invasion of the tubercle bacilli are caseation, liquefaction, fibrosis, calcification and ossification.
8) Caseation is tissue coagulation, consisting of
    a) Cell necrosis preceded by degenerative fat infiltration and
    b) Precipitation of fibrin and a homogenous hyaline-fibroid substance from the tissue fluids (vitreous degeneration). Caseation may occur rapidly and involve large areas simultaneously.

Virulence of mycobacteria

Mycobacterium tuberculosis (Mtb) adapts to persist in a nutritionally limited macrophage compartment. Lipoamide dehydrogenase (Lpd), the third enzyme (E3) in Mtb's pyruvate dehydrogenase complex (PDH), also serves as E1 of peroxynitrite reductase/ peroxidase (PNR/P), which helps Mtb resist host reactive nitrogen intermediates. [34]

Virulence is the ability of a microbe to cause disease in a host. It can also be defined as the degree of pathogenicity of a microbe.

Virulence is caused by several factors that are encoded by the bacteria. There are then several different ways by which the bacteria can cause the disease. First, the virulence factors can induce cell adhesion to the host cell. Second, they can increase the colonisation of the host body and the persistence. Third, they can favour invasion into host cells. Fourth, the bacteria can express inhibitors of the immune response. Fifth, some bacteria can express toxins.

***Mycobacterium tuberculosis* Virulence Factors [35]**

1. Secreted Factors

The first category is secreted factors, which are products that are exported outside the bacteria. They include culture filtrate proteins, such as HspX, Esat6/CFP-10, and 19-kD

2. Cell Surface Components

The cell surface components include proteins involved in synthesis of the cell wall.

3. Enzymes Involved in Metabolism

The third category includes enzymes involved in general or cellular metabolism. Some of these enzymes are involved in lipid and fatty acid metabolism, which allows bacteria to grow on fatty acids.

4. Transcriptional Regulators

The final category of virulence factors includes the transcriptional regulators.

Pathogenesis:

The portal entry of the organisms is inhalation, ingestion or inoculation. Inhalation is the most common route. Droplets containing MTB are released when a PT patient coughs. The primary site of infection depends on the mode of entry. The development of hypersensitivity plays a major role. T- Lymphocytes play a major role in conferring immunity against *Mycobacterium Tuberculosis*. [36]

T- Lymphocytes play a major role in conferring immunity against *Mycobacterium tuberculosis*. Tuberculosis bacillus circulates in blood and the number of circulating bacteria may reach high counts in immunocompromised hosts.

The host reaction to the bacilli which enter is initially exudative, and later proliferative. Initially the exudates are composed mainly of neutrophils. Later, lymphokine activated macrophages accumulate and these engulf the organisms. The pathological hallmark of tuberculosis is the tubercle which consists of an area of central caseation, around which there is infiltration by epithelioid cells, giant cells, round cells and peripherally by fibroblasts. Several such microscopic tubercles from the macroscopic tubercles which are seen in affected tissues. [37]

Clinical features:

Persistent cough, Haemoptysis, Pleural pain not associated with an acute illness, Spontaneous pneumothorax, Lethargy, Weight loss, Low grade evening fever and anorexia are the clinical features.[38] Physical examination of the chest may reveal signs of consolidation, cavitations, collapse, fibrosis, pleural effusion or pneumothorax.[39] As the condition proceeds the patient becomes emaciated. Cough with expectoration is pronounced and extreme cachexia develops (Phthisis). If left untreated, death occurs due to extensive disease, cachexia, intercurrent infection or any of the following complications. [40]

Complications [41]

Pneumothorax, pleural effusion, poncet's syndrome, secondary infection to cavities, pleural effusion, empyema, progressive fibrosis with dyspnea, aspergilloma, coexistence of apical tuberculosis with carcinoma and secondary amyloidosis

Investigations

These are the following investigations are available for tuberculosis [42]

- Pulmonary chest X-ray
- CT
- Bronchoscopy
- Gastric wash
- Transbronchial needle aspiration
- Endoscopic Ultrasound Needle Aspiration(EUS)
- PET
- Abreugraph
- Sputum analysis[43]
- Alternative sampling
- PCR
- Microscopic observation drug susceptibility assay culture

Treatment:

First line of anti-tuberculous drug [44]

- Ethambutol
- Isoniazid
- Pyrazinamide
- Rifampicin
- Streptomycin

Second line anti-tuberculous drug:

- Amino glycosides e.g: amikacin (AMK), kanamycin(KM)
- Polypeptides e.g: capreomycin, viomycin, enviomycin
- Fluoroquinolones e.g: ciprofloxacin, levofloxacin, moxifloxacin
- Thioamides e.g: ethionamide, prothionamide
- Cycloserine
- Terizidone

Third line anti-tuberculous drug :[45]

- Rifabutin
- Macrolides e.g.: clarithromycin
- Linezolid
- Thioacetazone
- Thioridazine
- Arginine
- Vitamin D
- Bedaquiline

Classification of Anti- TB drugs according to its pharmacological actions [46]

1. Tuberculocidal agents:

- Isoniazid
- Streptomycin
- Capreomycin
- Ciprofloxacin
- Rifampicin
- Pyrazinamide
- Kanamycin

2. Tuberculostatic agents:

- Ethambutol
- Ethionamide
- Thioacetazone
- Cycloserine

Though these anti- tubercular drugs have more therapeutic potentials, some adverse effects are always reported. Nausea, vomiting and epigastric pain, transitory and asymptomatic increase in hepatic enzyme levels, arthralgia, cutaneous pruritus or fever, psychosis, convulsive seizures, mental confusion and coma are some adverse effects are observed by anti-tubercular drugs.[47]

Drug Resistance

With over eight million cases and two million deaths annually, tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. Recently, it has been shown that multidrug-resistance (MDR) is the most important cause for mortality in patients with tuberculosis. Drug resistance is considered one of the main threats for tuberculosis control. Diabetes mellitus, prior tuberculosis treatment and intravenous drug use were risk factors for drug-resistant disease. The association between diabetes and drug-resistant TB should be further explored. Identifying clinical predictors of drug resistance can allow prompt identification of patients at risk for drug-resistant TB. [48]

Drug-resistant tuberculosis (TB) is a worldwide threat and constitutes an unparalleled challenge for disease control. Treatment of such patients is more complex, less effective, more toxic and much more expensive than treatment of patients infected with drug-susceptible TB strains.

International studies have identified various risk factors for drug-resistant TB; these include previous TB treatment, poor adherence to treatment regimens, inadequate regimens and positive smear result at the end of the second and third month of treatment. In addition of these programmatic factors, patients characteristics such as HIV co-infection, alcohol abuse and younger age are also believe to influence the drug resistance.

For instance, high prevalence levels of drug resistance have been found among HIV patients. In recent years, drug-resistant TB has emerged, largely due to delays in treatment, gaps in treatment protocol and ineffective or delayed drug-susceptibility testing. Multi-drug resistant tuberculosis (MDR-TB) is defined by the resistance of the bacillus to the most powerful TB drugs, Isoniazid and rifampicin, while extensively drug-resistant TB (XDR-TB) is also resistant to some second line drugs

So there is an urgent need of a drug which acts in MDR-TB and XDR-TB also.

3.3 PHARMACEUTICAL REVIEW

Purification processes in Siddha system (*Suddhiseithal)*

The process of detoxification or purification of the drug is called '*Suddhiseithal*' in Siddha medical terminology.

Nature has created innumerable plant, herbs, metals, poisonous substances, minerals, salts and other organic substances. Siddha had selected such of those things which can render relief to innumerable ailments of mankind suffered. Any matter in nature has to be utilized for medicinal purposes, the properties which may cause bad effects should be neutralized or eliminated. That's why every raw drug used in Siddha medicine is purified before preparing it as a medicine.

Purification

The exact part of the herb which has been described should alone be taken for medicine. There should not be other impurities like mud, sand or any such thing. If it is green leaf, dried or decomposed and insects infected leaves should be eliminated. Care should be taken in identifying the herbs properly.

As a general rule, when anything is subjected to be processed by using heat, soaking either alone or with some other substances, some chemical reaction may take place. In these process impurities, toxins would be eliminated and the substances become purified. Hence some of the poisonous herbs which are purified by using heat.

Importance of purification

The drugs when subjected to heat like roasting or soaked in liquids undergoes some chemical reactions. Such as oxidation of toxic substances to non-toxic substances, elimination of certain poisonous chemicals to non- poisonous substances. In these ways not only the toxicity, impurities are removed but also enhance the potency of the drugs.

Mathirai : [49]

The ingredients should be purified first and pounded into a fine powder and then macerated with the prescribed juices or liquid one after the other in their order. Each time it must be ground till it becomes waxy in consistency does not adhere to the fingers or mortar and pestle; finally it must be made into pills as prescribed size and dried in shade.

Other names : *Kuligai, Urundai*

Rule of tablet preparation

- Before preparing mathirai, the raw drugs required are powdered separately and then mixed to attain homogeneity, then ground together until it reaches waxy in consistency.
- The fine paste which does not stick to the mortar should be considered as the right consistency for rolling pills.
- If any of the preparation of pills, if mercury and sulphur are the key ingredients, mercury should be ground first and sulfur is added to it and ground and further continued.
- In case of inclusion of hard raw drugs, they should be ground first; the reason behind this if hard substances are added to smooth substances, they will not be ground finely.
- Croton seeds if included can be grinded at the last: because croton on grinding releases oil; if added early the effect of medicine will be suppressed, so it should be added finally.
- Aromatic substances like camphor, lac, musk are added just before 24 minutes the paste reaches waxy consistency.
- The paste should not adhere to the mortar nor does it adhere to the pestle.

Storage

They should be stored in well stopper glass vials, with relevant labels and instructions.

Shelf life of medicines

Medicines can be classified into internal and external medicines. They are each in 32 types. Mathirai comes under the category of internal medicines. The shelf life of medicines indicates the potency of medicines. The medicines even though seems to be fresh is not efficacious after sometime. So the medicines should not use after certain period.

The shelf life of *mathirai*,

As per Siddha literature *Agamarunthu padal* in *Gunapadam Thathu-seevam* text

"*உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி*
னுயிர்மூன்று திங்களெண்ணெய்
விரலிடுமு யர்ந்தமாத் திரைகடுகு பக்குவம்
.....
கொள்ளாறு மோராண்டு.....

\- *குணபாடம் தாது சீவம் வகுப்பு*

From above the quote the shelf life of Mathirai is 1 year. But according to Ayush guidelines the shelf life is 2 years.

Traditional tests for *Mathirai*

Characters

- Non sticky on rolling
- No cracks over the surface after drying
- Shall be rolled uniformly over the plane surface.

Based on these characters the drug is assessed as the appropriate one for medication.

Table 1: Testing parameters for *Mathirai* - AYUSH guidelines

S.No	Tests
1	Description, Colour, Odour
2	Weight Variation
3	Disintegration Time (Not more than 15 minutes)
4	Identification TLC/ HPTLC/GLC
5	Assay
6	Test for heavy/toxic metals Mercury Arsenic Cadmium Lead
7	Microbial Contamination Total Bacterial count Total Fungal count
8	Test for specific pathogen *Escherichia coli* *Salmonella species* *Pseudomonas aeruginosa* *Streptococcus aureus*
10	Test for aflatoxins B1, B2, G1, G2

Modern Aspect of the formulation:

Tablet (Pill)- *Mathirai*

A tablet is a pharmaceutical dosage form, it otherwise called as caplet. Medicinal tablets are called as "pills". Originally "pills" referred specifically to a soft mass rolled into a ball shape, rather than a compressed powder. [50]

As per Indian Pharmacopeia 2007 defined the tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are anticipated for oral route. A tablet consists an active medicament with excipents which are in powder form are compressed or pressed into a solid dosage form. About two third drugs prescribed are in solid dosage form and tablets include half of them.

Classifications:

As per IP2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007)

1. Uncoated Tablets:

This type of tablets contains single layer or more than one layer tablet consisting of active ingredient with the excipents, no additional cover is applied on to it after the compression.

2. Coated Tablets:

Coated types of tablets have an additional coating layer on it after the tablet was compressed, the coating layer of tablets formed with sugar, gums, resins, inactive or insoluble fillers, plasticisers, polyhydric alcohols, waxes.

3. Dispersible Tablets:

These are the film coated or uncoated tablets because a uniform dispersion when suspended in water

4. Effervescent Tablets:

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce CO_2, thus produced CO_2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release Tablets:

These types of tablets are the coated or uncoated tablets which are designed in such a way that the rate or location of the active ingredient released is modified. It includes enteric coated tablets, prolong release tablet or delay release tablet.

A) Enteric-coated Tablets:

These are also called as gastro resistant tablets as they resistant to the gastric juices; these are formulated by coating the tablet with anionic polymer of methylacrylic acid and their esters or by coating with cellulose acetyl pthylate.

E.g: erythromycin, NSAIDS

B) Prolonged- release Tablets:

These types are otherwise called as sustain release tablets or extended release tablets was formulated in such a way that the active ingredient is released for a prolong duration of time and is available in systemic circulation after administration.

C) Delayed-release Tablets:

This dosage form was planned to release the drug after some time or after the tablet has passed one part of the GIT into another. All enteric coated tablets are type of delayed action tablet but all delayed action of tablets was not enteric or not intended to produce enteric action.

6. Soluble Tablets:

These are coated or uncoated tablets which are planned to dissolve in water before they are administered.

7. Tablets for Use in the Mouth:

These are the tablet formulations which are planned to be show local action in the buccal cavity. These include buccal tablet, Sublingual Tablets and Troche or lozenges. Buccal tablets are placed in between the cheek and gingival. Sublingual tablets are placed below the tongue E.g: glyceryl trinitrate.

8. Tablets for other routes of administration:

These include implantable tablets and vaginal tablet. These are inserted in to the rectum or vagina for their local or systemic action.

Tablet Ingredients:

A tablet consist of active medicament with excipents which are in powder form are compressed or pressed into a solid dosage form. In addition to active ingredients, tablet contains a number of inert materials known as additives or excipents. [51]

- Diluent
- Binder and adhesive
- Disintegrents
- Lubricants and glidants

- Colouring agents
- Flavouring agents
- Sweetening agents

1. Diluent:

Diluents are fillers are used to make bulk of the tablet when the drug dosage itself was inadequate to produce the bulk these are used. Secondary reason is to provide better tablet properties such as improve cohesion, to permit use of direct compression manufacturing or to promote flow.

2. Binders and Adhesives:

These materials are added either dry or wet form granules or to form cohesive compacts for directly compressed tablet. Ex: Acacia, tragacanth Solution for 10-25% Concentration.

3. Disintegrents:

It added to the tablet formulations to facilitate its breaking or disintegration, when it contact in water in the GIT. Example: Starch 5-20% of tablet weight.

Super Disintegrents: Swells up to ten fold within 30 seconds when contact with water. Example: Crosscarmellose cross-linked cellulose.

4. Lubricant and Glidants:

Lubricants are planned to prevent adhesion of the tablet materials to the surface of dies and punches, reduce inter particle friction and may improve the rate of flow of the tablet granulation Example: Lubricants - Stearic acid, stearic acid salt - Stearic acid.

5. Coloring agent:

The use of colors and dyes in a tablet has three purposes:

- Masking off colour drugs
- Product Identification
- Production of more elegant product

All coloring agents must be approved and certified by FDA.

1. Flavoring agents:

For chewable tablet- flavour oil are used

2. Sweetening agents:

For chewable tablets: Sugar, Mannitol. Saccharine (artificial): 500 times sweeter than sucrose.

Advantages:

- These are very easy and handy to use.
- As these are unit dosage form fixed dose was administered.
- Modified drug release rate and duration of tablets be able to increase their therapeutic effect and increase the patient compliance by reducing the frequency of drug administration.
- These are cost-effective dosage forms when compared to other dosage forms
- The physical, microbial and chemical stability of tablet are superior to other dosage forms.

Disadvantages:

- The systemic availability of the drug depends on many physiological factors
- The onset of action was lesser when compared to Intra Venous route. (except sublingual tablets)
- Geriatric and infants cannot swallow the tablets easily.

3.4 PHARMACOLOGICAL REVIEW

PHARMACOLOGICAL SCREENING OF ANTI- TUBERCULOSIS [52]

a. Absolute concentration method (LJ)

A stock solution of active compound was prepared in sterile DMSO. Subsequent dilutions were made with sterile distilled water and aseptically added to the Lowenstein-Jensen media to give the desired final concentrations viz: 12.5, 25, 50, 100, 200 and 400µg/ml.

Preparation of Lowenstein-Jensen medium:

Ingredients of L-J medium

Potassium dihydrogen orthophosphate	
Anhydrous (KH_2PO_4)	: 2.4 gm
Magnesium sulphate anhydrous	: 0.24 gm
Magnesium citrate	: 0.6 gm
L-Asparagine	: 3.6 gm
Glycerol (reagent grade)	: 12.0 ml
Malachite green, 2% solution	: 20.0 ml
Distilled water	: To 600 ml

The ingredients were dissolved in 50ml-distilled water; after dissolving, glycerol malachite green solution was added and made up to 600ml with distilled water. This solution was autoclaved at 121^{o} C for 30 minutes to sterilize and cooled to room temperature and the solution was stored in refrigerator.

Preparation of egg solution

The clean hen eggs were broken individually into round bottomed flask and shaken vigorously to obtain homogenous solution and filtered through sterile gauze into a measuring cylinder upto 1 litre.

Preparation of complete medium

The following ingredients were aseptically pooled in a large, sterile flask and mixed well.

Mineral salt solution : 600ml

Homogenized egg solution : 1000 ml
(From 25-30 eggs, depending on size)

The active compound was mixed with the complete medium at different dilutions to obtain the necessary final concentrations. The medium was distributed in 6-8ml volumes in sterile universal containers and the caps tightly closed and inspissation is done at 85-90^{o}C for 50 minutes without delay to prevent sedimentation of heavier ingredients. After inspissation, the medium was incubated at 37^{o}C for 24 hours to check for sterility.

Standard bacterial suspension

Bacterial suspension was prepared by adding approximately 4 mg moist weight of a representative sample of the bacillary mass. Two to three loopfuls of culture were taken using the inoculation loop with 3mm internal diameter and added to 0.2 ml of sterile distilled water in a 7mm bottle containing twelve (2-3mm dia.) glass beads. To prepare a uniform suspension, 0.8ml sterile water was added and shaken. This suspension contained approx. 4mg/ml of the organism. For absolute concentration method this standard suspension was used.

Ten microliter of suspension was inoculated on both compound containing and compound free LJ slopes (control). Standard strain *M. tuberculosis* $H_{37}Rv$ was set up with each batch of test as assay control. These slopes were incubated at 37°C and read after 28 days. The MIC was calculated as the lowest concentration that inhibited growth (defined as 20 colonies or more on compound containing medium) as described in the Standard operating procedure of Mycobacteriology, NIRT (2010).

BRONCHODILATOR ACTIVITY IN ANIMAL MODELS

In vitro methods

Spasmolytic activity on guinea pigs isolated tracheal chain

The isolated tracheal chain of guinea pigs can be used for testing compounds which inhibit bronchospasm. It detects sympathomimetic, H_1-histamine receptor antagonistic properties of test drug.

Methodology

Guinea pig of either sex weighting between 300-500 gm are sacrificed using ether anesthesia. The entire trachea is dissected out and cut into individual rings. Twelve to fifteen rings are tied together with silk threads and mounted in the organ bath containing Krebs-Henseleit solution and maintained at 37^oC, under a tension of 0.5 gm and gassed with carbon. Isometric contractions are recovered via a strain gauge transducer on a polygraph. Forty five minutes are allowed for equilibration before the addition of the spasmogens. The following spasmogens used Histamine, Carbachol, LTC_4 or LTD_4. It takes about 10-12 min for reaching the contraction to a

maximum. At this standard and test drugs are administered. The bronchial response is allowed to plateau and recorded. The tissue is rinsed thoroughly and the control contractions are induced again by adding spasmogens. The percent inhibition of spasmogens induced contractions is calculated. From dose response curve ED_{50} is calculated. [53]

Isolated Frog Rectus Abdominis Muscle Preparation:

A frog is pithed and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally up to the anterior aspects of the limbs. This exposes the flat whitish muscle of the anterior abdominal wall from their pubic origin to their sternal insertion. The two recti are removed and placed in frog ringer solution in a shallow dish. They are carefully cleaned and one of them is trimmed to the desired size and mounted in an organ bath of 5ml capacity, at room temperature, aerated with oxygen. For recording purposes, an isotonic lever with a sideways writing point is used tangential to the smoked drum, balanced for a tension of 2.5gm with an extra load of 1gm on the long arm. A standard solution of Ach is added to the bath and a slow contraction is recorded on the slow moving drum for exactly 90 seconds. The drum is stopped and the bath fluid is replaced by fresh Frog-Ringer. An extra 1gm load is used to extend the muscle to its original length. [54]

In vivo methods

Histamine induced bronchospasm in guinea pig

Guinea pigs subjected to inhibition of aerosols containing histamine or other bronchospasm inducing agents, exhibits the symptoms of asphyxiating convulsions resembling acute attack of bronchial asthma. These challenging agents are administered in the form of aerosols through a nebulizer to individual guinea pigs placed in a histamine chamber. The initial symptoms are increased frequency of breathing, forced breathing and finally asphyxiating convulsions. The occurrence of these symptoms can be delayed by antagonistic drugs and bronchodilators. Pre-convulsion time is noted as the end point.

Methodology

Male guinea pigs weighing around 400 gm are used in groups of 8-10 animals. The animals are treated with test / standard drugs orally or subcutaneously. The animals are then placed in the standard Histamine chamber, 30 minutes after the administration of drug and exposed to an aerosol of 0.1 % solution of histamine dihydrachloride through a nebulizer. Time required for the onset of asphyxiating convulsions is recorded. The animal is immediately withdrawn from the inhalation box and placed in a well-ventilated area for revival from the convulsions. This method has been further improvised using an ultra-sound nebulizer which provides the steady exposure to histamine solution at a pre-determined rate. Percent of increase of pre-convulsion time is calculated from the average values of treated and control groups of guinea pigs. ED_{50} values denoting 50% increase in the pre-convulsion time can also be calculated. Histamine aerosol exposure is a very commonly used and dependable method for screening the bronchodilator activity of novel compound. [55]

Egg albumin induced anaphylaxis in guinea pig:

Guinea pig was sensitized by two intra peritoneal injections of 0.5 ml and 10% w/v solution of egg albumin at 48 hours interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and named as control group. Animals of group II received ethanolic extract trial drug (500 mg/kg. once daily) dissolved in distilled water for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms and mortality. [56]

ANTI-HISTAMINE ACTIVITY:

Effects of diphenhydramine in experimentally produced asthma in guinea pigs

Aim

To demonstrate the antagonistic effects of diphenhydramine against histamine induced bronchospasm in the guinea pig.

Principle

Guinea pig is very sensitive to histamine. When guinea pig is exposed to histamine vapour it exhibits bronchospasm, difficulty in breathing and convulsion. These effects of histamine are mediated through the action of histamine on H_1 receptors. Diphenhydramine is a H_1 receptors blocker. Therefore, diphenhydramine prevents the bronchospasm induced by histamine.

Equipments and other materials required

Histometer, stop watch, disposable needle and syringes.

Animal: Guinea pigs

Drug solutions required

1. Normal saline
2. Diphenhydramine 5 mg/ml
3. Histamine diphosphate 30µg/ml

Procedure

Select 4 guinea pigs having body weight between 250-350 gm, fast the guinea pigs for 12 hours before the experiment. Divide the guinea pigs into 2 groups of 2 animals each. Weigh the guinea pigs in each group and mark them for identification. Administer the drug solutions as Group I: Normal saline 1 ml/kg, Group II: Diphenhydramine 5 mg/kg. After one hour place each guinea pig in histamine chamber and replace the cover. With the help of compressor, spray a finely atomized mist of histamine diphosphate from nebulizer in both compartments. Using a stop watch record the time of histamine administration. Observe the signs of respiratory distress and the animal falling on its side and record the observations. [57]

Isolated Guinea Pig Ileum

Overnight fasted guinea pigs of either sex weighing 400-600gms were sacrificed using cervical dislocation method. The lower most 10cm of ileum was removed from the abdomen and placed in a shallow dish containing warm Triode solution. Ileum lumen was cleaned by passing through warm 0.9% saline and then segments about one inch in length, were made. The mesenteric attachment and blood etc. were

carefully cleaned and tissues was mounted in a thermostatically controlled Dale's organ bath containing 20ml Triode's solution under basal tension of 500mg. The composition of solution is Nacl, 137; Cacl2, 1.8; Kcl, 2.7; glucose, 5.55; NaHco3, 11.9; Mgcl2, 1; NaH2Po4, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on a student physiography using isotonic transducer, which exerted a basal tension equivalent to 500mg load tissue. The issue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. Increasing concentration of histamine were added to the bath and the control cumulative concentration-response curve was constructed.[58]

LATERAL RESEARCH:

1. *Cinnamomum verum / Cinnamomum zeylanicum:*

ANTIMICROBIAL ACTIVITY:

The objective of this work was to evaluate the chemical composition, antimicrobial and antileishmanial activities of *Cinnamomum zeylanicum*, *Origanum vulgare* and *Curcuma longa* essential oils. Chemical analysis was performed by gas chrromotography- mass spectrometry. Antimicrobial activity was performed by disk diffusion and minimum inhibitory concentration (MIC) test.

Result:

The disc diffusion method showed that the largest halo was observed for *Cinnamomum zeylanicum* essential oil against *Escherichia coli*, whereas *Origanum vulgare* and *Curcuma longa* essential oil presented better inhibition against *Staphylococcus aureus*. [59]

ANTI-HYPERTENSIVE ACTIVITY:

Background:

The aqueous extract of the stem bark of *Cinnamomum zeylanicum* possesses anti- hypertensive and vasodilatory properties. The present work investigates the acute and chronic antihypertensive effects of the methanol extract of *Cinnamomum zeylanicum* stem bark (MECZ) in L-NAME-induced hypertensive rats.

Results

Acute intravenous administration of *Cinnamomum zeylanicum* extract (5, 10 and 20 mg/kg) to L-NAME-induced hypertensive rats provoked a long-lasting decrease in blood pressure. Mean arterial blood pressure decreased by 12.5%, 26.6% and 30.6% at the doses of 5, 10 and 20 mg/kg, respectively. The MECZ also significantly lower the plasma level of triglycerides (38.1%), total cholesterol (32.1%) and LDL-cholesterol (75.3%) while increasing that of HDL-cholesterol (58.4%) with a significant low atherogenic index (1.4 versus 5.3 for L-NAME group).[60]

2. *Cinnamomum tamala:*

ANTIDIABETIC, ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITIES :

Abstract

The ethanol extract of the leaf of *Cinnamomum tamala* was used for investigating its effect against diabetes using a rat model. Oral administration of the ethanol extract of *Cinnamomum tamala* resulted in significant decrease in the blood glucose levels, glycosylated haemoglobin, thiobarbituric acid reactive substances and serum lipids in diabetic rats under investigation. It was also observed that the oral administration of ethanol extract of *Cinnamomum tamala* significantly decreased the levels of reduced glutathione and superoxide dismutase in the hepatic and renal tissues of alloxan-induced diabetic rats. These results provided evidence for the antidiabetic, antioxidant and antihyperlipidemic effects of the ethanol extract of *Cinnamomum tamala* when administered orally.

Result:

Preliminary phytochemical screening of the aqueous (hot and cold) and ethanol extracts of *Cinnamomum tamala* showed the presence of various phytoconstituents including alkaloids, steroids, terpenoids, flavonoids and saponins. Several phenolics, flavonoids and alkaloids possess marked antidiabetic activities. Saponins possessed hypocholesterolemic and antidiabetic properties. The presence of these phytochemicals supports the claim for the medicinal uses of *Cinnamomum tamala* as potent antioxidant and hypoglycaemic agent.[61]

3. *Eleterria cardamomum:*

ANTI-ANXIETY ACTIVITY:

Abstract:

The present study aims to evaluate the efficacy of *Eleterria cardamomum* methanolic extract on anxiety-like behavior in a rat model of PTSD. Adult male Wistar rats (200–250 gm) were used in this study. The rats underwent single prolonged stress (SPS) or control and intraperitoneally received either saline or different dosages (200, 400, and 800 mg/kg) of *Eleterria cardamomum* methanolic extract before and after stress sessions. Findings demonstrated that *Eleterria cardamomum* methanolic extract, particularly at the dose of 400 mg/kg, significantly ($P < 0.05$) improved anxiety-like behavior in a rat model of PTSD, as examined by the open field, elevated plus-maze, and rotarod tests. Administration of *Eleterria cardamomum* methanolic extract after stress might help to prevent the formation of anxiety-like behavior in the animals.[62]

4. *Piper longum:*

IMMUNOMODULATARY AND ANTITUMOR ACTIVITY:

Abstract:

Alcoholic extract of the fruits of the plant *Piper longum* and its component piperine was studied for their immunomodulatory and antitumor activity. Alcoholic extract of the fruits was 100% toxic at a concentration of 500 μg/ml to Dalton's lymphoma ascites (DLA) cells and 250 μg/ml to Ehrlich ascites carcinoma (EAC) cells.

Result:

Administration of *Piper longum* extract and piperine increased the total WBC count to 142.8 and 138.9%, respectively, in Balb/c mice. The number of plaque forming cells also enhanced significantly by the administration of the extract (100.3%) and piperine (71.4%) on 5th day after immunization. Bone marrow cellularity and α-esterase positive cells were also increased by the administration of *Piper longum* extract and piperine.[63]

5. *Karkandu* (Sugar candy):

ANTIOXIDANT ACTIVITY:

Abstract:

Phenolic compounds in sugar cane (*Saccharum officinarum* L.) juice were identified and quantified by analytical high performance liquid chromatography and photodiode array detection, showing the predominance of flavones (apigenin, luteolin and tricin derivatives), among flavonoids and of hydroxycinnamic, caffeic and sinapic acids, among phenolic acids, representing a total content of around 160 mg/L. A tricin derivative was present in the highest proportion (>10% of the total).

Result:

The phenolic extract obtained from sugar cane juice showed a protective effect against *in vivo* MeHgCl intoxication and potent inhibition of *ex vivo* lipoperoxidation of rat brain homogenates, indicating a potential use for beneficial health effects and/or therapeutic applications.[64]

6. Dried dhiratchai (Vitis vinifera):

Abstract

Six raisin grape cultivars and 10 new raisin grape selections were analyzed for antioxidant activity (ABTS assay) and for total and individual phenolic compounds. Samples were freeze–dried and values are reported on a dry weight basis. Antioxidant activity across the 16 samples ranged from 7.7 to 60.9 μmol Trolox/g DW, with A95-27 exhibiting the greatest activity. Total phenolic content, determined in gallic acid equivalents using the Folin–Ciocalteau assay, ranged from 316.3 to 1141.3 mg gallic acid/100 g DW and was strongly correlated ($r = 0.990$) with antioxidant results. Concentrations of individual phenolics were determined by HPLC. *trans*-Caftaric acid was the predominant compound in all samples. A95-15 contained the lowest concentration (153.5 μg/g DW) of caftaric acid, while Fiesta contained the highest concentration (598.7 μg/g DW). Selections A56-66, A95-15, and A95-27 had much higher levels of catechin (86.5–209.1 μg/g DW) and epicatechin (126.5–365.7 μg/g DW) than the other samples.[65]

7. Adhimathuram (Glycyrrhiza glabra):

Objective:

To study the anticonvulsant activity of ethanolic extract of *Glycyrrhiza glabra* in albino rats and mice. **Methods:** The anticonvulsant activity of ethanolic extract of roots and rhizomes of Glycyrrhiza glabra (10, 30, 100 and 500 mg/kg, i.p.) in mice was assessed using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using albino mice. The lithium-pilocarpine model of status epilepticus was also used to assess the anticonvulsant activity in rats. **Results:** The ethanolic extract of *Glycyrrhiza glabra* did not reduce the duration of tonic hindleg extension in the MES test even in the dose of 500 mg/kg. However, the extract significantly and dose-dependently delayed the onset of clonic convulsions induced by pentylenetetrazol. The dose of 100 mg/kg afforded protection to all animals. The extract also protected rats against seizures induced by lithium-pilocarpine. Conclusion: The ethanolic extract of *Glycyrrhiza glabra* inhibits PTZ and lithium-pilocarpine-induced convulsions but not MES-induced convulsions. Anticonvulsant *Glycyrrhiza glabra* lithium-pilocarpine MES PTZ A.[66]

8. KARCHURKAI (Phoenix dactilifera):

Phoenix dactylifera exhibits potent anti-oxidative properties both *in vitro* and *in vivo*. This allows the fruit to prevent depletion of intrinsic protection from oxidative cell damage and assist these defense systems in reducing cell damage. Macroscopically, this mechanism may be relevant to the prevention of various adverse drug events common to chemotherapy including hepatotoxicity, nephrotoxicity, gastrotoxicity and peripheral neuropathy. While such effects have only been studied in small animal systems, research suggests a potential application to more complex mammalian systems and perhaps a solution to some problems of chemotherapy in hepato-compromised and nephro-compromised patients.

4. MATERIALS AND METHODS

4.1 PREPARATION OF THE DRUG

Selection of drug

The trial drug "*Thirijadhathi mathirai"* will be prepared as per Siddha literature *"Anubava vaithiya devaragasiyam."*

Ingredients

- *Lavangapattai* (*Cinnamomum verum*) - ½ thola (6g)
- *Lavangapathiri (Cinnamomum tamala*) - ½ thola (6g)
- *Elakkai (Eletteria cardamomum*) - ½ thola (6g)
- *Thipilli (Piper longum*) - 2 thola (24g)
- *Karkandu* (Sugar candy) - 4 thola (48g)
- Dried *Dhiratchai (Vitis vinifera)* - 4 thola (48g)
- *Adhimadhuram* (*Glycyrrhiza glabra*) - 4 thola (48g)
- *Karchurakai* (*Phoenix dactilifera)* - 4 thola (48g)

Collection of the drugs

The crude drugs such *as Lavangapattai, Lavangapathiri, Elakkai, Thipilli, Karkandu, Dried dhiratchai, Adhimathuram, Karchurakai* were procured from *Ramasamy Chettiar* Raw Drug Stores at Chennai.

Identification and Authentication

All raw drugs were identified and authenticated by the experts from Gunapadam department (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai.

The specimen samples of the identified raw drugs were preserved in the laboratory of P.G *Gunapadam* for future references.

PURIFICATION OF THE DRUGS: [67]

All the drugs were purified as per the Siddha literature.

Lavangapattai

Dried in sunlight.

Lavangapathiri

Dried in sunlight.

Elakkai

Roasted in the pan and outer skin was removed.

Thipilli

Soaked in 60 ml lime juice until the juice is evaporates.

Karkandu

Clean the dust particles.

Dried dhiratchai

Clean the dust particles and allowed it to dry.

Adhimathuram

Cleaned and cut into small pieces and dried.

Karchurkai

The seed is removed and dried in sunlight.

Method of preparation

All the ingredients were purified. The purified drugs are crushed into fine powder separately, then mixed together. The ingredients are triturated with honey in a stone mortar until it reaches the waxy consistency. Then rolled into tablet in size of *siru nellikai allavu* (5gm), dried in shade and labeled as *Thirijadhathi mathirai (TM).*

Preservation

The medicine was preserved in a clean, air tight container.

Administration of the drug

Form of the medicine	: Chewable tablet
Route of administration	: Enteral
Dose	: 1 to 2 tablets
Time of administration	: Twice a day
Adjuvant	: Hot water.
Indication	: ***Kabakasam***, *Thummal, Suram.*

Fig. 9 Ingredients of Thirijadhthi mathirai

Lavangapattai
(Cinnamomum verum)

Lavangapathiri
(Cinnamomum tamala)

Elakkai

(Eletteria cardamomum)

Thipilli

(Piper longum)

Karkandu

(Saccharum officinarum)

Adhimadhuram

(Glycyrrhiza glabra)

Dhiratchai

(Vitis vinifera)

Karchurakai

(Phonex dactilifera)

Thirijadhathi mathirai

4.2 STANDARDISATION OF THE DRUG

World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care. The process of evaluating the quality and purity of herbal drugs by means of various parameters like physical, chemical and biological observation is called standardization. Standardization of the this drug comes under the following categories

- Physico-chemical analysis
- Phyto chemical analysis
- Bio chemical analysis

ORGANOLEPTIC EVALUATION

The Organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, size etc.

1. Colour examination:

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and wrote in results.

2. Odour examination:

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of TM was noted in results table.

4.2.1 PHYSICO-CHEMICAL INVESTIGATION

Physico-chemical studies like total ash, water insoluble ash, acid Insoluble ash, loss on drying at 105°C and pH were done at, Tamilnadu Dr. M.G.R. Medical University, Chennai.

1. pH value

Potentiometrically pH value was determined by a glass electrode and a pH meter. The pH of the *TM* was written in results column.

2. Loss On Drying:

An accurately weighed 1gm of ***Thirijadhadhi mathirai*** formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

3. Determination of total ash:

Weighed accurately 2gm of ***Thirijadhadhi mathirai*** formulation was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

4. Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

5. Determination of water soluble ash:

Total ash 1gm was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

6. Determination of water soluble Extractive:

5gm of air dried drug, coarsely powered ***Thirijadhadhi mathirai*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrate was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

7. Determination of alcohol soluble extractive:

1 gm of air dried drugs, coarsely powdered ***Thirijadhadhi mathirai*** was macerated with 20 ml. alcohol in closed flask for 24 hours. With frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.[68]

4.2.2 PHYTO CHEMICAL EVALUATION

Phytochemical are chemical compounds that are naturally present in plants. Phytochemical screening of the plant gives a vast idea about the chemical constituents present in the drug.

The preliminary phytochemical screening test was carried out for each extracts of ***Thirijathathi mathirai*** as per the standard procedure.[69]

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Dragendroff's Test:

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test:

Filtrates were hydrolyzed with dilute HCL, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides:

Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides.

a) Modified Borntrager's Test:

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

5. Detection of phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

6. Detection of tannins Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

7. Detection of Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

8. Detection of proteins

a) Xanthoprotein Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

9. Detection of aminoacids

a) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

10. Detection of diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

11. Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

12. Test for Quinones:

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

13. Test for Fixed oils and Fats:

a. Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

The Preliminary phytochemical studies of aqueous extract of **Thirijathathi mathirai** were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of **Thirijathathi mathirai.**

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference. [70]

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument	: CAMAG (Switzerland).
Sample Applicator	: Camag Linomat - IV applicator with N_2 gas flow.
Photo documentation System	: Digi store - 2 documentation system with Win Cat & video scan software.
Scanner	: Camag HPTLC scanner - 3 (030618), Win Cats - IV.
Development Chamber	: Camag HPTLC 10X10, 10 X 20 twin trough linear Development chamber.
Quantity applied	: 5, 10 µl for extracts and 5 µl for standards
Stationary phase	: Aluminum plate pre-coated with silica gel 60(E. Merck)
Plate thickness	: 0.2 mm.
Mobile Phase	: For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).
Scanning wavelength	: 575 nm
Laboratory condition	: 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

4.2.3 BIO-CHEMICAL ANALYSIS[70]

The bio-chemical analysis was done to identify the acid and basic radicals present in the *TM*.

Preparation of extract

5gm of *TM* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary Basic and Acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the *TM* , 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *TM* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of *TM* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *TM* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *TM*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous)**

The *TM* extract, was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *TM* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *TM* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *TM* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of *TM* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *TM* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *TM* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *TM* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *TM* extract, 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *TM* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. **Test for Phosphate**

The *TM* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The *TM* extract was treated with conc.Hcl and observed for the appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of *TM* extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the *TM*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.4 MICROBIAL ACTIVITY

BACTERIAL LOAD: [71]

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

1. *Escherichia coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)

3. *Pseudomonas aeroginosa* (ATCC 27853)
4. *Klebsiella pneumoniae* (ATCC 13883)

PROCEDURE

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

ANTI-FUNGAL LOAD:[71]

AGAR- WELL DIFFUSION METHOD

PRINCIPLE:

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED:

1. Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39 gm of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)

3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- Aspergillus niger (ATCC 16404)

PROCEDURE:

Potato Dextrose agar plates were prepared and overnight grown species of fungus, Aspergillus niger were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).

4.2.5 SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy [72]

Fig. 11. FTIR INSTRUMENT

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganics. Examples include paints, adhesives, resins, polymers, coatings and **drugs**. FTIR is an effective analytical instrument for detecting functional groups.

Applications:

- Quantative scans
- Qualitative scan solids, liquids, gasess
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›):

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.

- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

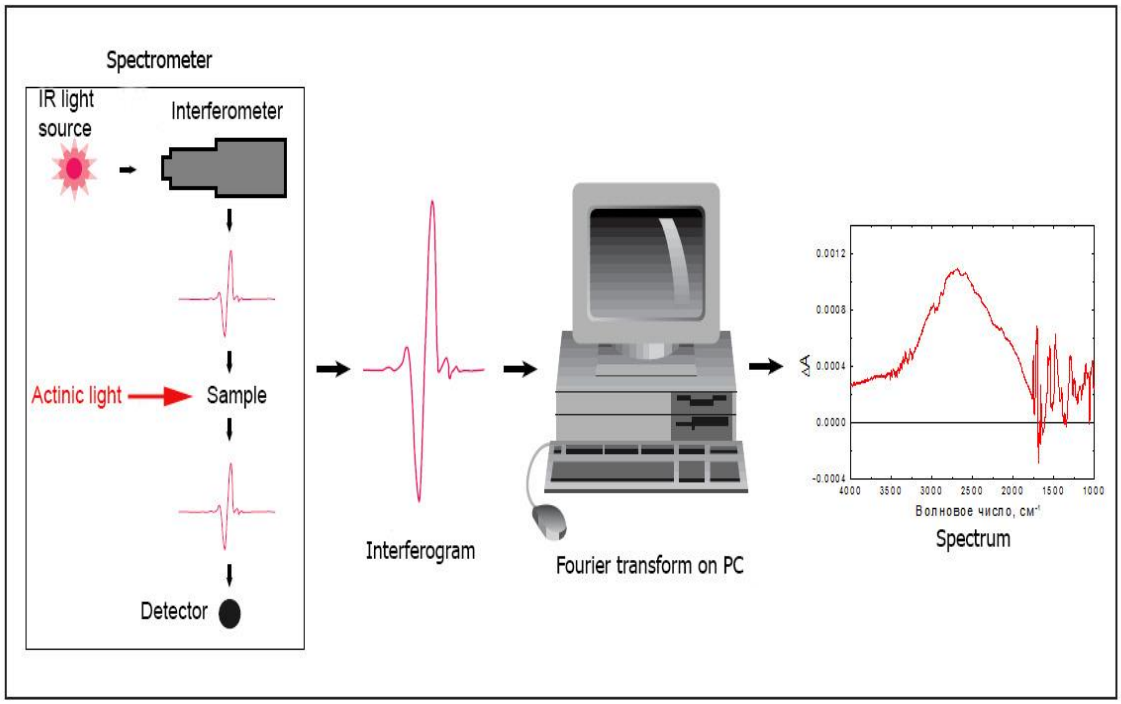


Fig. 12.FTIR MECHANISM

SEM (SCANNING ELECTRON MICROSCOPE)[73]

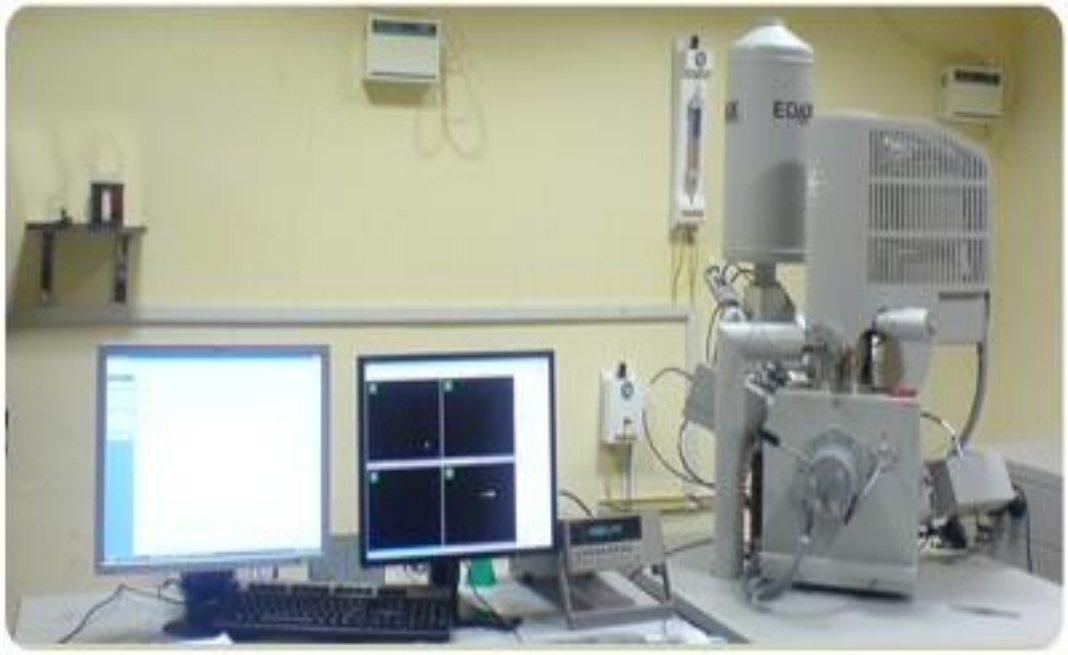


Fig. 13 SEM INSTRUMENT

Definition:

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM analysis applications

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture)

- Chemical composition (when used with EDS) Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal

SEM Analysis with EDS – qualitative and semi-quantitative results

Magnification – from 5x to 300,000x

Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height

Materials analyzed – solid inorganic materials including metals and minerals.

The SEM analysis process

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyze the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on

interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- Back scattered electrons
- Characteristic x-rays light
- Specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

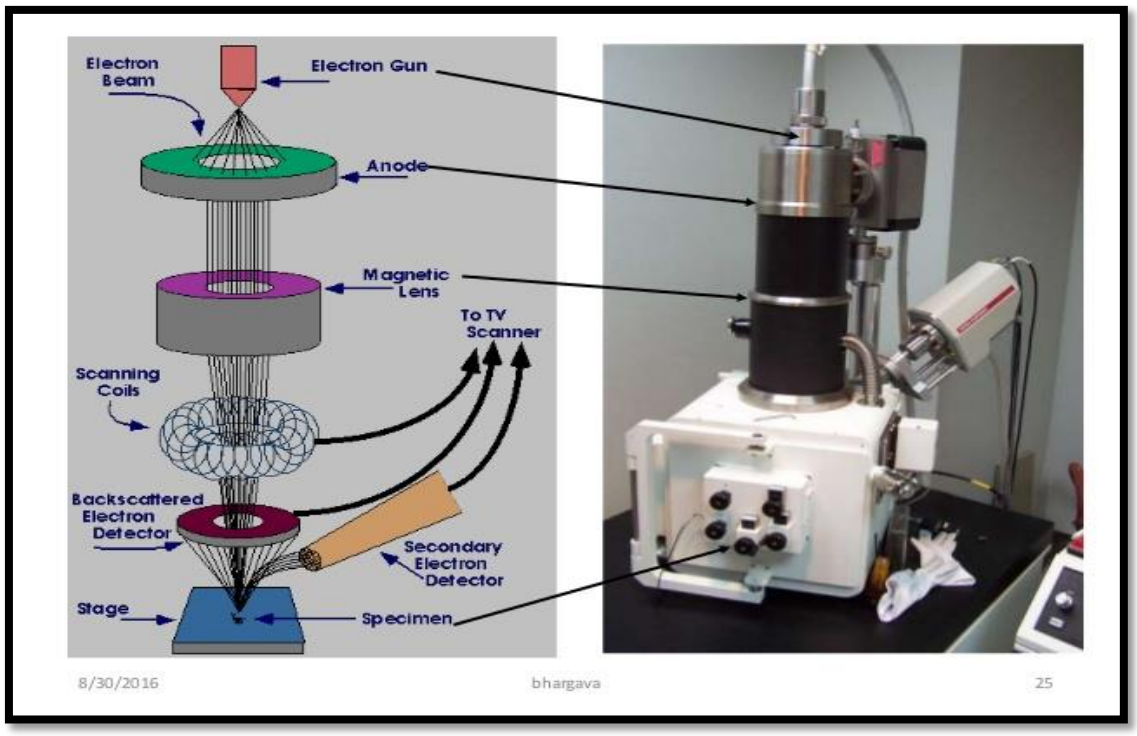


Fig. 14 SEM MECHANISM

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig. 15 ICP-OES INSTRUMENT

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle:

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES. [74]

Application:

The analysis of major and minor elements in solution TM.

Objectives:

- ❖ Determine elemental concentrations of different metals.
- ❖ Learn principles and operation of the ICP-OES instrument
- ❖ Develop and put on a method for the ICP-OES sample analysis
- ❖ Enhance the instrumental conditions for the analysis of different elements
- ❖ Probes the outer electronic structure of atoms

Mechanism:

In plasma emission spectroscopy (OES), a TM solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values.[75]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

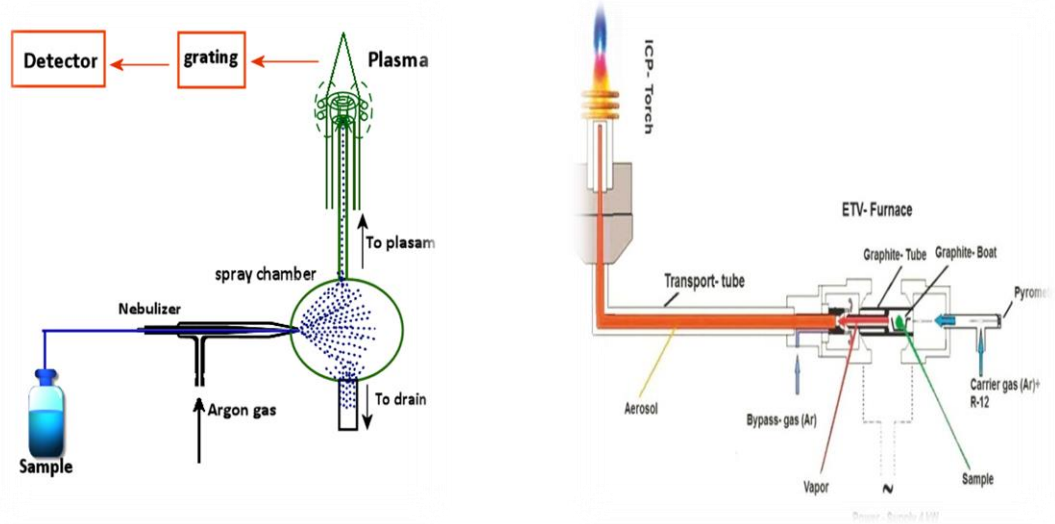


Fig. 16 ICP-OES MECHANISM

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby TM are introduced in liquid form for analysis.

100 mg TM was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested TM solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106

4.2.6 TOXICOLOGICAL STUDIES

ACUTE ORAL TOXICITY – OECD GUIDELINES - 423

- Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423
- The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (IAEC approved number: 05/32/PO/RE/S/01/CPCSEA dated 12/10/2018)
- These studies were conducted in C.L. Baid Metha College of Pharmacy, Thuraipakkam, and Chennai**.**

PRINCIPLE:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e. no further testing is needed, dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgement with respect to classifying the test substance to one of a series of toxicity classes. [76]

METHODOLOGY:

Selection of animal species:

The preferred rodent species are mouse and rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within ± 20 % of the mean weight of any previously dosed animals.

Housing and feeding conditions:

The temperature in the experimental animal room should be 22ºC (±3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation of doses:

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, i.e. at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal.

In rodents, the volume should not normally exceed 1ml/100g of body weight: however in the case of aqueous solutions 2 ml/100g body weight can be considered. With

respect to the formulation of the dosing preparation, the use of an aqueous solution/ suspension/ emulsion is recommended wherever possible, followed in order of preference by a solution/ suspension/ emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

EXPERIMENT PROCEDURE:

Administration of doses:

THIRIJADHATHI MATHIRAI suspended in 2% CMC with uniform mixing and was administered to the groups of wistar Albino rats in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16-18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels:

Three animals are used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was most likely to produce mortality in some of the dosed animals. The available information suggests that mortality is likely at the highest starting dose level 300 mg/kg body weight, so the trial or limit test was conducted. The time interval

between treatment groups is determined by the onset, duration and severity of toxic signs.

Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be toxic, i.e., having toxicity even below regulatory limit doses. A limit test at one dose level of 50 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

OBSERVATIONS:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Observations include changes in skin, fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded.

Body weight:

Individual weight of animals was determined before the test substance was administered and at least weekly thereafter. Weight changes was calculated and recorded. At the end of the test surviving animals were weighed and humanely killed.

Pathology:

All the test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours also was considered.

DATA AND REPORTING:

All data were summarized in tabular form showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility and necropsy findings.

Test substance and Vehicle:

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *THIRIJADHATHI MATHIRAI* with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle:

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique.

Test animals and Test conditions:

Female Wistar Albino rats (more than 100 gms) were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai and maintained in the animal laboratory of C.L.Baid Metha college of Pharmacy,Chennai. All the animals were kept under standard environmental condition (23±2°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore). Rats were deprived of food but not water (16-18 hours) prior to administration of the test drug. The principles of laboratory animal care were followed and the Department's ethical committee approved the use of the animals and the study design. [77]

REPEATED DOSE 28-DAYS SUB-ACUTE ORAL TOXICITY STUDY OF PPR ON WISTAR ALBINO RATS (OECD – 407 GUIDELINES):

Test Substance	**:** THIRIJADHATHI MATHIRAI
Animal Source	: Animal house of King Institute of Preventive medicine
Animals	: Male and Female Wistar Albino rats
Age	**:** More than 8 weeks
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior to and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking on fur.
Diet	**:** Pelleted feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles ad libitum.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** Between 20 to 24°C,
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** Each of 12 hours.

Justification for Dose Selection:

The results of acute toxicity studies in wistar Albino rats indicated that *THIRIJADHATHI MATHIRAI* was nontoxic and no behavioural changes was observed upto the dose level of 300 mg/kg body weight. On the basis of the results of acute toxicity study, the doses selected for the study were15mg/kg (x), 30mg/kg (5x) and 60 mg/kg (10x) body weight. The oral route was selected for use because the oral route is considered to be a proposed therapeutic route.[78]

Preparation and administration of dose:

THIRIJADHATHI MATHIRAI at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 15, 30 and 60 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Functional Observations:

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals at fasting over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene is used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations:

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included heart, kidneys, liver and spleen of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multicomparision test using a computer software programme GRAPH PAD 8.1 version.

4.2.7 PHARMACOLOGICAL STUDIES:

EVALUATION OF ANTI- TUBERCULOSIS ACTIVITY:

Mycobacterial strains

Three Mycobacterial strains, viz., standard strain of M. tuberculosis H37Rv, clinical were used for this study. This strain was grown and maintained on Lowenstein Jensen (L-J) medium in the Centre for drug discovery and development, Sathyabama Institute for Science and Technology, Chennai, Tamil Nadu, India.

Anti-Mycobacterial activity using Luciferase Reporter Phage assay

Antitubercular activity is studied against standard laboratory strain *Mycobacterium tuberculosis* H37Rv by adopting Lusiferase reporter phage assay. About 350µL of G7H9 broth supplemented with 10% albumin dextrose complex and 0.5% glycerol was taken in cryovials and added with 50µL of crude extract in order to get the final concentration of 250µg/mL and 500µg/mL. One hundred microliter of Mycobacterium tuberculosis cell suspension was added to all the vials. DMSO (1%) was also included in the assay as solvent control. All the vials were incubated at 37ºC for 4 hours. After incubation, 100µL from each vial was transferred to luminometer cuvette. About 100µL of D-luciferin was added and relative light unit (RLU) was measured in luminometer. RLU reduction by 50% or more when compared to control was considered as having antitubercular activity.[79]

EVALUATION OF BRONCHODILATOR ACTIVITY: HISTAMINE INDUCED BRONCHOCONSTRICTION IN GUINEA PIG:

Overnight fasted guinea pigs were divided into six groups each containing 6 animals.

- Group I was treated as control,
- Group II received standard drug Salbutamol (2 mg/kg).
- Group III, IV *THIRIJADHATHI MATHIRAI* (200 and 400mg/kg).

All the doses were given orally once a day for 5 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsive time (PCT) was determined from the time of exposure to onset of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air. Group 2 received Salbutamol. These animals were again subjected to histamine aerosol after 1hour of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula[1, 2].

Percentage Protection = (1 - T1/T2) X 100 Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.[80,81]

EVALUATION OF ANTI-HISTAMINE ACTIVITY:

ANTI- HISTAMINE AND ACETYLCHOLINE INDUCED BRONCHOSPASM IN GUINEA PIGS

The method was followed in which bronchospasm was induced in guinea pigs by exposing them to histamine aerosol under standardized conditions. The efficacy of the test drug was evaluated by estimating the degree of protection imparted against histamine induced bronchospasm.

Experimental bronchospasm:

Bronchospasm was induced by exposing the animals to histamine acid phosphate 0.25% and acetylcholine chloride 10% under a constant pressure 40 mm/Hg from the inbuilt nebuliser of the histamine chamber

The animals were placed inside the “Histamine exposition chamber” and a finely atomized mist of 0.25% solution of histamine acid phosphate was blown into the chamber through a nebulizer under 40mm Hg pressure.

The preconvulsive time (PCT) was determined from the time of exposure to the onset of dyspneoa leading to the appearance of convulsions which is known as preconvulsive dyspnoea (PCD). As soon as the PCD were noted, the animals were removed from the chamber and placed in fresh air.

The sharp fall of animal due to asphyxia was taken as the end-point. The time period from the onset of the exposure to the sharp fall of the animal on its side was designated as the "exposition time".

The animals which did not develop the typical features of asphyxia like restlessness, irritation of skin and mucous membrane (sneezing), scratching of ear, deep slow and labored respiration, drawing of the abdominal wall, to and fro movement of the head, appearance of cyanosis, air hunger (animal raising its head in intense inspiratory effort) even after more than three minutes of exposure to histamine were considered resistant or completely protected (C.P) and such animals were excluded from study.

Two and half hour after the initial exposure to histamine aerosol, single dose of the drug/ vehicle was administered to various groups as per the following schedule:

Grouping and drug treatment:

Group I: Control group, received Normal Saline 1ml/100gm.

Group II: Treated with Chlorpheniramine maleate 1.6mg/kg (standard drug).

Group III and IV: Treated with TM 400mg/kg and 800mg/kg respectively

All the drugs/ vehicle was administered orally. The doses of the test drug were selected after observing the response to it in pilot studies as well as after referring to the literature. The dose of Chlorpheniramine maleate were chosen after considering their recommended clinical doses.

These animals were subjected to histamine and acetylcholine challenge one and a half hours after receiving the drug and the PCT was noted. Animals which withstood exposure to histamine or acetylcholine aerosol for 15 minutes were considered to be completely protected.

Data analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Probability values of 0.05 or less were considered statistically significant.[82,83,84]

5. RESULTS AND DISCUSSION

The medicine *Thirijadhathi mathirai* had been subjected to various studies and standardization to establish the works of *Siddhars* to be true. Literary collections, Physicochemical Studies, Elemental analysis, Bio-chemical analysis, Toxicity studies, Pharmacological studies are done to prove the Anti-tuberculosis activity of *Thirijadhathi mathirai*. Literary collections about the drug from various text books give hope about its activity. The studies strongly supported the fact through these results. They are discussed below:

The extensive review on botanical aspect gave information about the Microscopical, Macroscopical, medicinal uses, Chemical constituents and the importance of the herbs in detail. Most of the herbs included in the formulation are Anti-bacterial, Anti-microbial, Expectorant, Anti pyretic and Tonic**.**

Cinnamomum zeylanicum posses anti-microbial activity which is useful in the treatment of *Mycobacterium tuberculosis*. The major compound cinnamaldehyde posses strong antimicrobial activity.[86]

Cinnamomum tamala *is* considered as potential antimicrobial agent and posses anti-bacterial activity.[87]

Eletteria cardamomum also cures asthma, bronchitis, cold and cough. It also has strong anti-bacterial activity.[88]

Thipilli is indicated for pulmonary tuberculosis, chronic cough, asthma for its bronchodilator activity. Piperine present in this will exhibits Anti-bacterial activity, has significant activity against multi drug resistant strains of mycobacterium spp. [89]

Karkandu has antibiotic action.[90]

Dried dhiratchai is used to treat anaemia; it can be given for the patients with fever, cough and tuberculosis.

Adhimathuram has tonic and expectorant action. It will melt phlegm due to *Iyam*. Exhibits activity against MDR isolates of H_{37}Rv *Mycobacterium tuberculosis* strain.[91]

Karchurkai posses antimicrobial activity. It also has immunostimulant activity which enhances the immune system.

The pharmacological aspect of the drug says about their mode of action which was used from ancient times. The current pharmacological methods available for carrying out the Anti-tuberculosis activity were explained clearly and the suitable In-vitro models for carrying out the activities were discussed. And explains, whether the drug effectively inhibits the proliferation of *Mycobacterium tuberculosis*.

The Pharmaceutical review explained the preparation of *Mathirai* in detail including the purification of raw drugs, methods of manufacturing and the *Siddha* parameters for the standardization of *Mathirai.*

The standardization of the drugs was achieved through various procedures like analyzing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters is described below.

Results of physical characterisation:

Table: 2 Organoleptic Character

S.No	**Parameters**	**Results**
1	Colour	Brown
2	Odour	Characteristic odour
3	State of matter	Semi Solid
4	Consistency	Soft
5	Shape	Spherical

Table: 3 Results of Physico-chemical analysis

S.NO	**Parameters**	**Results**
1	pH	4.69
2	Total ash	2.84%
3	Acid insoluble ash	0.49%
4	Water soluble ash	1.42%

5	Water soluble extraction	48.8%
6	Acid soluble extraction	44%
7	Loss on Drying at 105°C	8.65%

DISCUSSION:

pH value

The pH of *TM* is 4.69. It is acidic in nature. For drug absorption to occur, a drug must cross biological barriers. Drugs that are weak acids will pick up a proton when placed in an acidic environment and will, thus, be un-ionized.

Drugs that are un-ionized will be better able to diffuse through a lipid cellular membrane, cross a biologic barrier and enter the bloodstream.

A drug that is a weak acid will be absorbed primarily in the acidic environment. Eventhough the stomach is acidic, it is not well-suited for drug absorption, even for drugs that are weak acids due to its thick mucus layer and relatively small surface area. As a result, acidic drugs are most to be absorbed in the acidic areas of the proximal duodenum.[92]

Total ash

Total ash value will determine the amount of total inorganic content (ammonium, potassium, calcium, chloride, iron etc.) present in the drug after ignition is measured through it.

The total ash value of TM is 2.84%, this indicates that the drug has less contamination and adulteration.

Acid insoluble ash

The acid insoluble ash value of the drug denotes the amount of siliceous matter (dust, sand etc,) present in that drug.

The quality of the drug is better if the acid insoluble ash value is low.

Here, acid insoluble ash value of TM is 0.49%. Hence, it represents the superior quality of the TM.

Water soluble ash

Water soluble ash is a part of total ash value, which denotes the diffusion capacity of the drug.

Here, the water soluble ash value of TM is 1.42%, which represents easy facilitation of diffusion and osmosis mechanism.

Disintegration time

According to Ayush guidelines, the disintegration time does not exist more than 45 min.

The disintegration time of TM is 22 min.

This implies a reasonable disintegration time, thereby a better absorbability and solubility is achieved.

Loss on Drying (LOD)

It indicates the amount of volatile substance and moisture present in the drug.

The loss on drying percentage of TM is 8.65. This indicates the stability and shelf life of the drug.

PHYTOCHEMICAL ANALYSIS:

Table: 4 phytochemical screening tests

S.No.	Phytochemicals	Test Name	H2O Extract
1	Alkaloids	Dragendroff's Test	+ve
3	Glycoside	Modified Borntrager's Test	+ve
4	Saponin	Froth Test	+ve
5	Phenols	Ferric Chloride Test	+ve
7	Flavonoids	Alkaline Reagent Test	+ve
8	Proteins	Xanthoprotein Test	-ve
10	Diterpenes	Copper Acetate Test	+ve
11	Gum & Mucilage	Extract + Alcohol	+ve

+ve/-ve present or absent if component tested

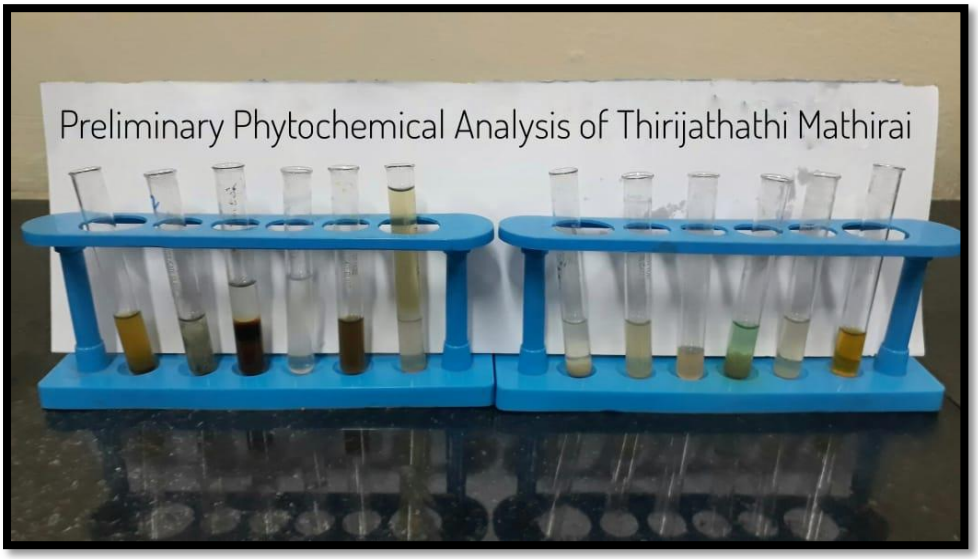


Fig. 17 Phytochemical analysis

DISCUSSION:

From the primary phytochemical screening tests of *Thirijadhathi mathirai* shows Alkaloids, Glycosides, Saponnins, Flavanoids, Phytosterol, Phenol, Diterpenes, Gum and mucilage.

Alkaloids [93]

- Alkaloids have a wide range of pharmacological activity like Anti-bacterial.
- These alkaloids will enhance the action of TM.
- Alkaloids possess antispasmodic, analgesic, bactericidal effects.
- Alkaloids are the active principles producing many essential effects in protecting the body.

Glycosides

- Many plants store chemicals in the form of inactive glycosides, such plant glycosides are used as medications.
- Inhibitory effect against *Mycobacterium tuberculosis* showed the conjugates of glycosides. The anti-tubercular activity is higher than drug Pyrizamide but lower than the drug Isoniazid.[94]

Tannins

- Tannins exhibits anti- Mycobacterial activity by inhibiting the total growth and also of patients strain, which was fully sensitive to the standard anti tuberculosis drug.[95]
- Herbal preparation containing tannins are used for bronchitis and many others.
- Tannins are considered as anti-oxidants which prevents the onset of degenerative diseases.[96]

Phenols

- Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (anti-oxidant, anti-inflammatory effects etc,)[97]
- It will reduce the inflammation in tuberculosis.
- Phenols could be a source of natural anti- tuberculosis agents.

Diterpenes

- Suppress the inflammatory process.
- Anti-oxidant and Anti-microbial activity.
- Anti-bacterial agents will destroy the bacterial growth and proliferation of bacteria (*Mycobacterium tuberculosis*).[98]
- The substance inhibited 99% the growth of *Mycobacterium tuberculosis* H_{37}Rv, that oleanolic acid has a synergistic effect when combined with Isoniazid, Rifampicin or Ethambutol.[99]

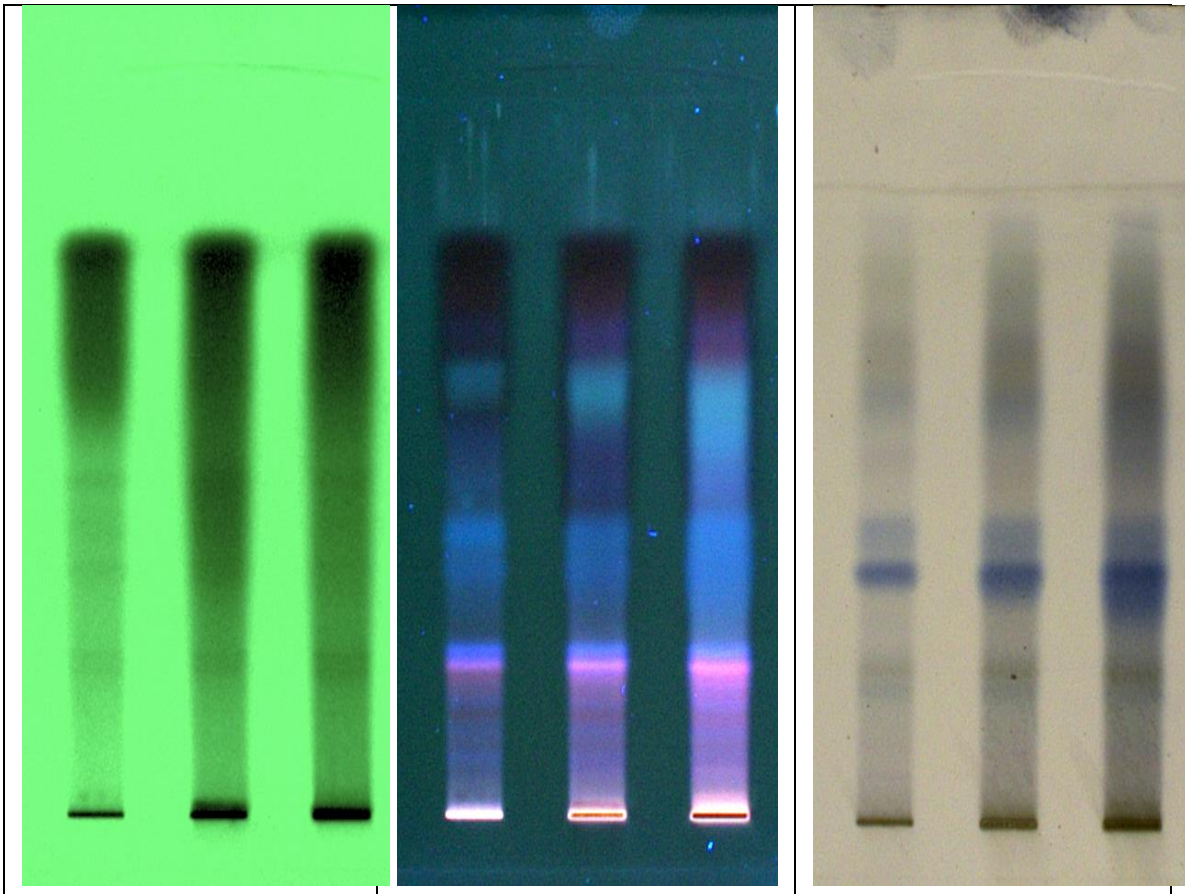
Proteins

- Body uses protein to build and repair tissues, which are degenerated by the tubercular bacilli.[100]
- Substituted aminopyrimidine protein kinase B (PknB) inhibitors show activity against *Mycobacterium tuberculosis*.[101]

TLC/ HPTLC finger print studies

Stationary Phase - Silica Gel 60 F_{254}

Mobile Phase - Toluene: Ethyl Acetate: Formic Acid (5: 2: 0.1 v/v/v)



λ = 254 nm	λ = 366 nm	λ = 575 nm (Derivatized)

Fig:18 HPTLC finger print

Table: 5 TLC photo documentation of chloroform extract of *Thirijadhathi Mathirai*

Colour	R_f value(s)	Colour	R_f value(s)	Colour	R_f value(s)
Green	0.21	Magenta	0.21	Light Blue	0.21
Green	0.22	Blue	0.22	Brown	0.22
Green	0.34	Light Blue	0.34	Blue	0.34
Green	0.40	Light Blue	0.40	Light Blue	0.42
Green	0.46	Greenish Blue	0.60	Violet	0.52
Green	0.78			Light Blue	0.60

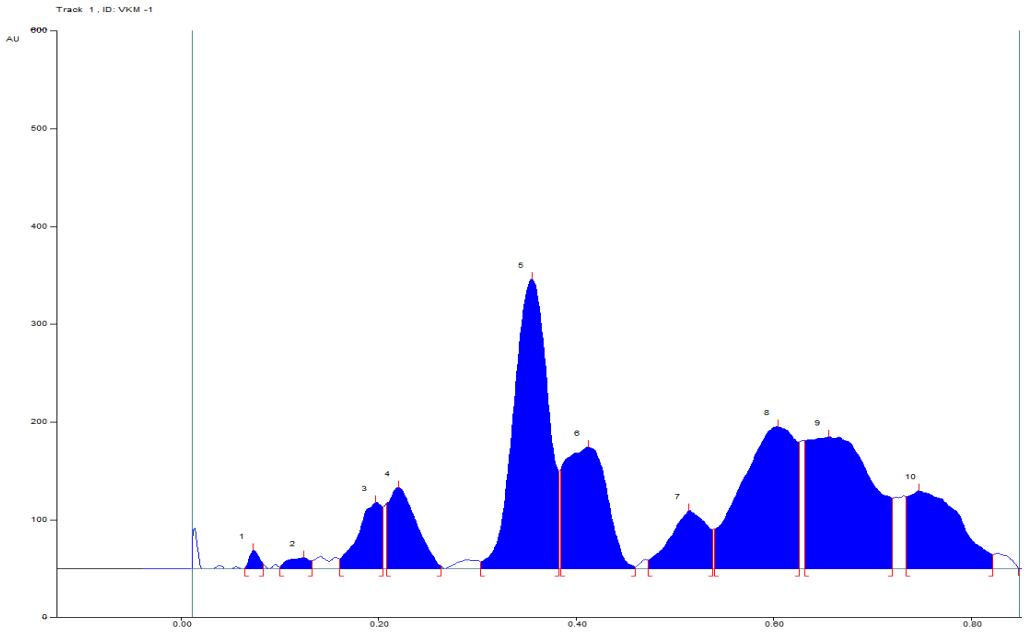


Fig: 19 Derivatized plate HPTLC Chromatogram @ 575 nm

Table: 6 Peak Table @ 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.3 AU	0.07 Rf	18.5 AU	1.81 %	0.08 Rf	5.4 AU	171.4 AU	0.43 %
2	0.10 Rf	1.9 AU	0.12 Rf	11.2 AU	1.10 %	0.13 Rf	8.0 AU	233.7 AU	0.59 %
3	0.16 Rf	10.0 AU	0.20 Rf	67.9 AU	6.65 %	0.21 Rf	63.5 AU	1515.2 AU	3.84 %
4	0.21 Rf	66.8 AU	0.22 Rf	83.2 AU	8.15 %	0.26 Rf	2.9 AU	2132.0 AU	5.40 %
5	0.30 Rf	7.1 AU	0.36 Rf	296.5 AU	29.05 %	0.38 Rf	99.8 AU	9309.5 AU	23.59 %
6	0.38 Rf	100.7 AU	0.41 Rf	124.3 AU	12.18 %	0.46 Rf	1.8 AU	4864.1 AU	12.33 %
7	0.47 Rf	8.9 AU	0.52 Rf	59.2 AU	5.80 %	0.54 Rf	40.1 AU	2010.2 AU	5.09 %
8	0.54 Rf	40.5 AU	0.61 Rf	145.4 AU	14.24 %	0.63 Rf	29.6 AU	7288.9 AU	18.47 %
9	0.63 Rf	131.3 AU	0.66 Rf	134.8 AU	13.21 %	0.72 Rf	72.4 AU	8092.8 AU	20.51 %
10	0.74 Rf	73.8 AU	0.75 Rf	79.7 AU	7.81 %	0.82 Rf	14.3 AU	3840.5 AU	9.73 %

DISCUSSION:

A qualitative fingerprinting of *Thirijadhathi mathirai* has been performed by HPTLC method, which provides qualitative insights into the bioactive Constituents present in the drug. HPTLC shows separation of components present in the Chloroform extract of *Thirijadhathi mathirai*. The method may be applied to identify the *Thirijadhathi Mathirai* from other manufacturing process.

The present study revealed that *Thirijadhathi mathirai* showed best results in Toluene: Ethyl Acetate: Formic acid: 5:2:1 solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and 575 nm and visible light range, best results were shown at 575 nm.

TLC plate showed different colour phyto constituents of chloroform extract of *Thirijadhathi mathirai.* The bands revealed presence of six greenish, five light blue, two blue, one greenish blue, one magenta, one brown and one violet band showing the presence of steroids, terpenoids, alkaloids, flavanoids, tannins, lignans and saponins.

The results from HPTLC finger print scanned at wavelength 575 nm for chloroform extract of *Thirijadhathi mathirai*. There are ten polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.06 to 0.74 in which highest concentrations of the phyto constituents was found to be 29.05% and 14.24 % with its corresponding Rf value were found to be 0.30 and 0.54 respectively.

BIO CHEMICAL ANALYSIS:

Table : 7 Results of acid and basic radical studies

S.No	Parameter	Observation	Result
1.	Test for Potassium	Formation of yellow colour precipitate	Positive
2.	Test for Ammonium	Appearance of brown colour	Positive
3.	Test for Phosphate	Appearance of yellow precipitate	Positive

From the acid and basic radicals studies of *Thirijadhathi mathirai* presents the following chemicals: Potassium, Ammonium and phosphate.

Potassium:

- Clavulanate potassium demonstrates synergestic anti-bacterial activity.[103]

Ammonium :[104]

- Ammonium is essential in the body as a building block for making proteins and other complex molecules.
- It is essential for much biological process and serves as a precursor for amino acid and nucleotide synthesis.

Phosphate: [105]

- Phosphate is an essential mineral primarily used for growth and repair of body cells and tissues.
- It is also required for a variety of biochemical processes including energy production and pH regulation.
- It is a component of DNA and RNA molecules.
- Hemoglobin, the important oxygen carrying protein in the bloodstream, also depends upon phosphorus contained in its structure for proper function.
- In TB patient, it will produce energy to the patient, regulate the pH and repairs the host cell.

Table: 8 ANTI-MICROBIAL ACTIVITY:

BACTERIAL LOAD:

GRAM POSITIVE

Table: 8.1 Organism: *Staphylococcus aureus*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Thirijadhari Mathirai (TM)	Streptomycin (100µg)	26
	250	17
	500	21
	1000	23

14mm- low sensitive; 15mm- moderate; 16mm- highly sensitive

GRAM NEGATIVE

Table: 8.2 Organism: *Escherchia coli*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Thirijadhari Mathirai (TM)	Streptomycin (100µg)	26
	250	14
	500	20
	1000	22

14mm- low sensitive; 15mm- moderate; 16mm- highly sensitive

Table: 8.3 Organism: *Klebsiella pneumoniae*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Thirijadhari Mathirai (TM)	Streptomycin (100µg)	25
	250	15
	500	19
	1000	23

14mm- low sensitive; 15mm- moderate; 16mm- highly sensitive

Table: 8.4 Organism: *Pseudomonas aeroginosa*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Thirijadhari Mathirai (TM)	Streptomycin (100µg)	30
	250	17
	500	24
	1000	26

14mm- low sensitive; 15mm- moderate; 16mm- highly sensitive

Table: 8.5 Organism: *Aspergillus niger*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Thirijadhari Mathirai (TM)	Clotrimazole(100µg)	37
	250	24
	500	26
	1000	32

14mm- low sensitive; 15mm- moderate; 16mm- highly sensitive

Inference:

1. *Escherchia coli* - Highly sensitive in 500(µg/mL)
2. *Klebsiella pneumoniae* - Highly sensitive in 500 (µg/mL)
3. *Pseudomonas aeruginosa* - Highly sensitive in 250(µg/mL)
4. *Staphylococcus aureus* - Highly sensitive in 250 (µg/mL)
5. *Aspergillus niger* - Highly sensitive in 250 (µg/mL)

DISCUSSION:

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial and anti-fungal agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *TM* was compared with Standard drug Streptomycin and Clotrimazole (100µg)/ml disc for the following pathogens, they are *Escherchia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Aspergillus niger*. The results represents *TM* potentially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm –

Moderate, above 16 mm – Highly sensitive. The findings reveal that the Siddha drugs *TM* have anti-microbial potency against bacterial and fungal pathogens which is used in the treatment of diseases.

INSTRUMENTAL ANALYSIS:

Fig. 20 FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)

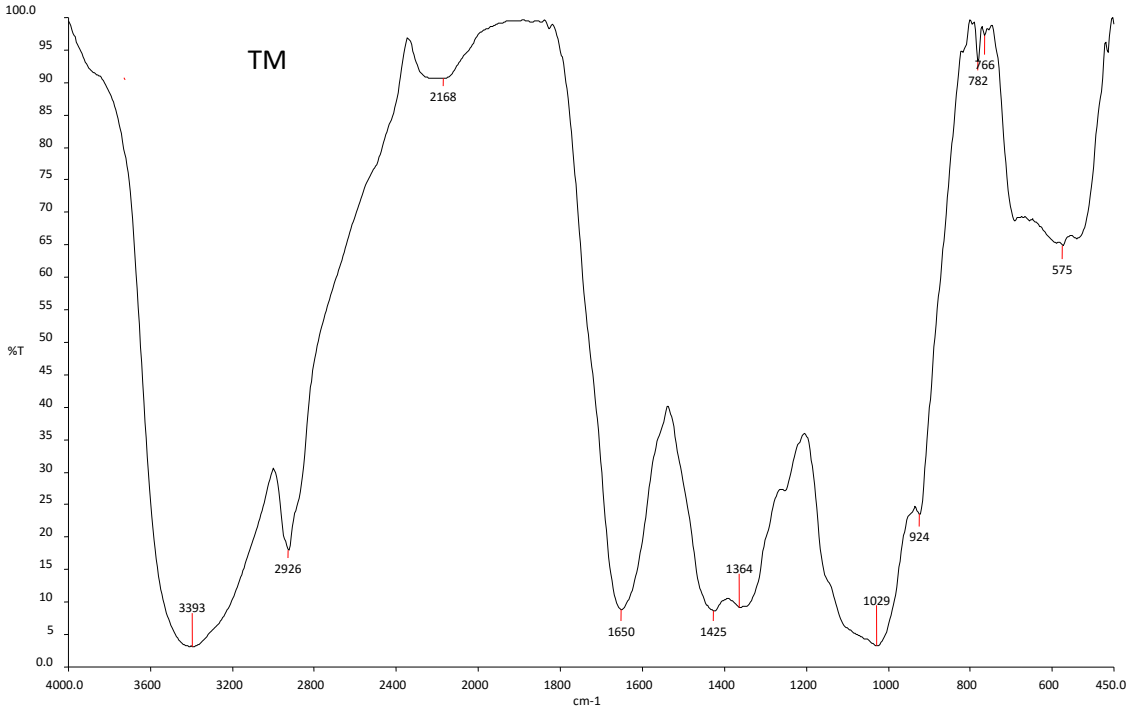


Table: 9 FTIR interpretation of *Thirijadhathi mathirai*

Absorption peak cm-1	**Stretch**	**Functional Group**
3393	O-H(stretch, H- Bonded), N-H stretch,	Amide and alcohols
2926	C-H stretch, O-H stretch	Alkenes and Acid
2168	-C ≡C- stretch	Alkynes
1650	C=C stretch, C=O stretch	Alkenes, Amide
1425	-C-H bending, C=C stretch	Alkanes, Aromatic

1364	-C-H bending, C-F stretch, N-O stretch	Alkanes, Alkyl Halide, Nitro group
1029	C-F stretch, C-O stretch	Alkyl Halide, Ether
924	=C-H	Alkenes
782	=C-H bending, C-Cl Stretch	Alkenes, Alkyl Halide
766	=C-H bending, C-Cl Stretch	Alkenes, Alkyl Halide
575	C-Br Stretch	Alkyl Halide

DISCUSSION:

In TM there will be Amide, Alcohol, Alkanes, Alkenes, Alkyl Halide and Nitro group.

Amide

Some antibiotics are made with amides (E.g Ampicilline) to treat bacterial infection. [106]

Alcohol

Ethanol killed the tubercle bacilli in sputum or water suspension within 15 seconds. [107]

SEM (SCANNING ELECTRON MICROSCOPE)

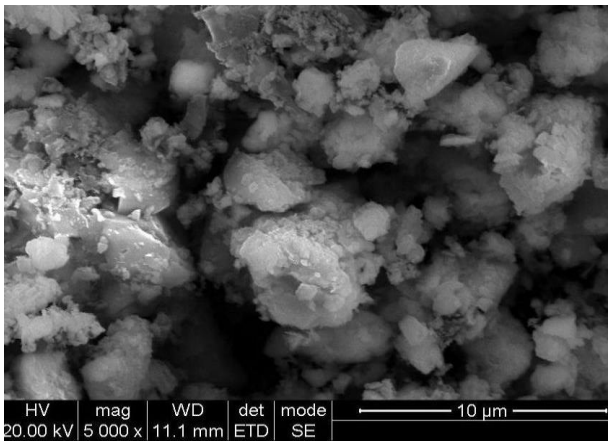


Fig: 21 SEM image

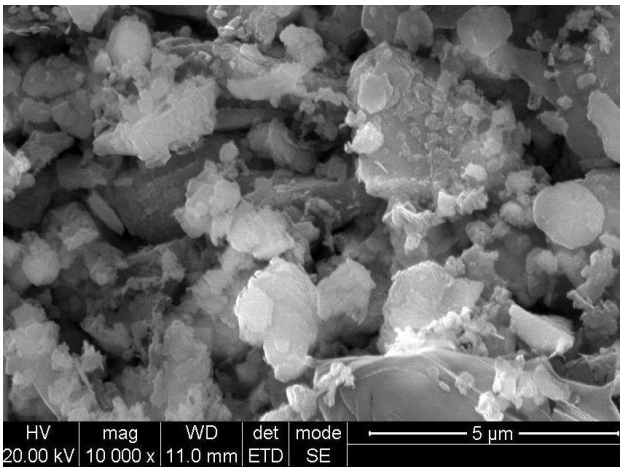


Fig: 22 SEM image

DISCUSSION:

Micro medicine has its benefit in the treatment for many chronic diseases. The advantage of the micro particle is its smaller size which enhances the solubility, bioavailability of the drug and avoids macrophage clearance. It reduces possibly of side effects.

Particles ranging from 1 to 1000 nm are called micro particles. SEM analysis of the drug *Thirijadhathi mathirai* revealed the presence of micro particles of size 2 to 4 microns. The particles of size micron show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

ICP-OES RESULTS OF *THIRIJADHATHI MATHIRAI*

Table: 10 ICP-OES interpretation of *Thirijadhathi mathirai*

S. no	Elements	Wavelength	Detected levels
1.	Arsenic (As)	188.979	BDL
2.	Calcium (Ca)	315.807	21.180 mg/L
3.	Cadmium (Cd)	228.802	BDL
4.	Iron (Fe)	238.204	01.334 mg/L
5.	Mercury (Hg)	253.652	BDL
6.	Potassium (K)	766.491	13.571 mg/L
7.	Sodium (Na)	589.592	24.180mg/L
8.	Nickel (Ni)	231.604	BDL

9.	Lead (Pb)	220.353	BDL
10.	Phosphorus (P)	213.617	96.357mg/L
11.	Aluminium (Al)	396.152	BDL
12.	Copper (Cu)	327.393	BDL
13.	Magnesium (Mg)	285.213	01.774mg/L
14.	Zinc (Zn)	206.200	01.256mg/L

BDL:Below Detectable Limit

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The toxic metals and the permissible limits

Heavy metals	**WHO & FDA limits**
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

DISCUSSION:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

ICP-OES reveals the concentration of many minerals present in the drug. It also has physiologically important minerals like Calcium, Iron, Potassium, Sodium, Phosphorus, Magnesium and Zinc. *Thirijadhathi mathirai*, the heavy metals like Arsenic, Cadmium, Lead and trace element like Nickel were below detectable level. This reveals the safety of the drug so that it can be used in long terms without side effects.

TOXICOLOGICAL STUDIES

Acute oral toxicity in rats

Dose finding experiment and its behavioural Signs of Toxicity for *Thirijadhathi mathirai*

Table: 11 Observation of acute toxicity study

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Normal
6	Salivation	Normal	6	Salivation	Normal
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table: 12 Dose finding experiment and its behavioural Signs of Toxicity for *Thirijadhathi mathirai*

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000mg/kg	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6.Touch Response 7. Decreased Motor Activity 8.Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16. *Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality*

Table: 13 Observation of Body weight

DOSE	**DAYS**		
	1	7	14
CONTROL	320.2±42.30	322.4 ± 60.10	323.6 ±52.10
HIGH DOSE	302.4± 1.21	314 ± 2.04	321.2 ± 2.10
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table: 14 Water intake (ml/day) of Wistar albino rats group exposed to *Thirijadhathi mathirai:*

DOSE	**DAYS**		
	1	7	14
CONTROL	58 ± 1.02	58±9.20	59.4±1.04
HIGH DOSE	59.4±2.20	59.8±3.40	59.9±6.24
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table 15: Food intake (gm/day) of Wistar albino rats group exposed to *Thirijadhathi mathirai*

DOSE	DAYS		
	1	7	14
CONTROL	61.04±2.62	62.2±4.76	64.3±6.26
High DOSE	69.4±4.23	70.4±6.22	76.6±4.18

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

DISCUSSION:

In the acute toxicity study, the rats were treated with different concentration of *Thirijadhathi mathirai* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioural changes and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.

These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

In acute toxicity test the *Thirijadhathi mathirai* was found to be nontoxic at the dose level of 2000mg/ kg body weight.

SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Table: 16 Body weight (g) changes of rats exposed to *Thirijadhathi mathirai*

Dose (mg/kg/day)	*Days*				
	0	**7**	**14**	**21**	**28**
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	125.92±0.63	128.14±1.01	131.19±1.30	133.82±1.18	138.62±1.27
200	126.49±3.6	128.66±3.2	130.42±4.67	134.12±6.52	135.32±3.08
400	128.53±3.83	135.17±1.34	139.30±1.35	141.34±1.58	146.55±1.50

Values are expressed as mean ±S.E.M. N=10

Table 17: Water intake (ml/day) of Wistar albino rats group exposed to *Thirijadhathi Mathirai*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	60.1 ± 8.72	60±1.52	60.2±1.40	61±1.32	61.4±1.62
LOW DOSE	65.1±1.21	65.6±4.22	66.6±1.02	65.2±2.06	66.4±1.20
MID DOSE	62.1±1.02	62.3±1.21	62.1±2.62	63.4±4.32	63.4±1.64
HIGH DOSE	64.1±1.81	64.2±1.32	64.4±1.14	64.6±1.62	65.8±2.02
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 18: Food intake (gm/day) of Wistar albino rats group exposed to *Thirijadhathi Mathirai*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	34±4.14	34.2±6.12	34.3±2.18	34.2±1.14	34±5.62
LOW DOSE	36.3±1.64	36.3±1.51	36.2±1.51	36.5±1.62	36.5±1.22
MID DOSE	34.1±2.12	34.2±3.50	34.2±2.14	34.2±2.16	35.2±1.64
HIGH DOSE	32.4±1.62	32.1±1.64	32.6±2.36	32.6±1.20	36.4±2.32
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table: 19 Effect of *Thirijadhathi mathirai* on Haematological parameters in rats

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
RBC (x 10^6/mm^3)	7.51 ± 0.16	6.46±0.32	7.4±1.46	7.32±0.9
PCV (%)	48.2 ± 1.3	43.3±2.12	48.2±1.62	47.6±3.37
Hb (%)	15.6 ± 0.19	14.8±2.46	14.9±3.6	14.56±1.46
WBC (x 10^3/mm^3)	10.12 ± 1.2	11.5±1.6	11.7±2.1	12.8±4.16
Neutrophils (%)	22 ± 4	26.46±5.3	34.2±4.2	28.7±2.78
Mononuclear cells (%)	76 ± 2	72.4±3.8	60.4±2.3**	70.3±4.2
Eosinophils (%)	2.4 ± 0.6	2.2±0.28	1.6±0.06	1.8±0.07
Platelets (x 10^3/mm^3)	423.2 ± 48.8	424.56±16.4	446.14±32.6	414.8±28.44

*Values are expressed as mean □ S.E.M (Dunnett's test). *P<0.05, **P<0.01,***P<0.001 vs control; N=10*

Table: 20 Effect of *Thirijadhathi mathirai* on Biochemical parameters in rats

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Protein (g/dl)	8.62 ± 1.3	6.9±0.94	8.6±0.56	7.18±0.78
Albumin (g/dl)	4.8 ± 0.6	4.4±0.34	3.7±0.54	4.22±0.76
BUN (mg/dl)	19.2 ± 1.2	21.2±1.4	24.81±4.6	22.7±2.42
Urea (mg/dl)	64.24 ± 3.11	66.42±5.6	68.3±4.2	66.53±4.4
Creatinine (mg/dl)	0.82 ± 0.16	0.46±0.02*	0.72±0.06	0.83±0.08
Total Cholesterol (mg/dl)	91.24 ± 1.35	96.5±7.9	117.14±8.2*	103.7±6.24
Triglycerides (mg/dl)	50.15 ± 3.21	59.3±4.4	69.14±3.12**	70.18±4.43**
Glucose (mg/dl)	110.16 ± 8.62	97.67±5.18	117.8±5.26	83.94±7.16*
Total Bilirubin (mg/dl)	0.205 ± 0.04	0.242±0.06	0.533±0.07**	0.753±0.04
SGOT (U/L)	73 ± 2.4	53.2±4.4*	66.8±2.84	72.19±7.38
SGPT(U/L)	28.4 ± 1.2	33.6±3.46	59.7±6.69**	71.6±6.52**
Alkaline phosphatase(U/L)	102.4 ± 3.6	96.7±6.6	103.6±4.9	86.44±3.23
Sodium (mEq/L)	138.12 ± 3.14	112.7±2.6	136.42±7.32	119.44±5.82*
Potassium (mEq/L)	7.2 ± 1.34	3.26±0.18	5.42±0.28	4.63±0.09*

*NS- Not Significant, **(p >0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

DISCUSSION:

The dose selected for the sub-acute toxicity study was 100mg, 200mg/kg and 400mg/kg of *Thirijadhathi mathirai*.

All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

There was no significant changes were observed in haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

DISCUSSION ON HISTOPATHOLOGY:

Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for haematological and blood chemical analyses were taken from common carotid artery.

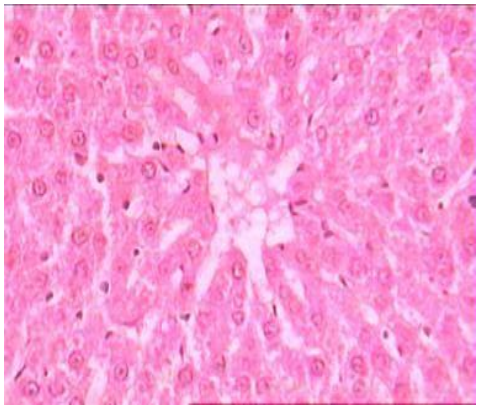
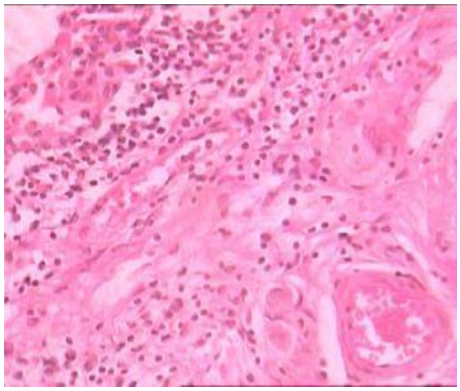
All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

From the acute and sub-acute toxicity studies the drug produced some significant changes, but the values were found within normal limits. So the drug *Thirijadhathi mathirai* was nontoxic and safe.

FIG. 23 HISTOPATHOLOGY SLIDES:

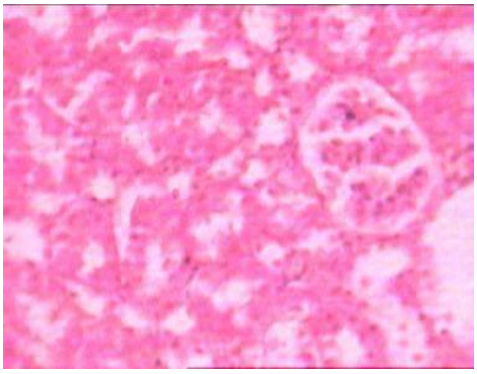
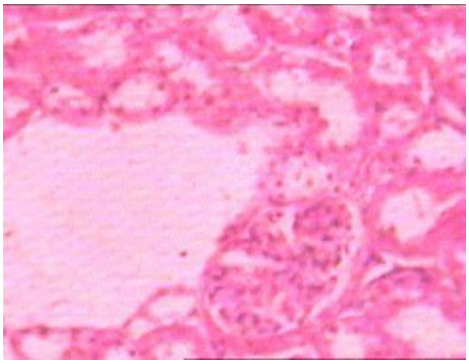
LIVER:

NORMAL **HIGH DOSE**

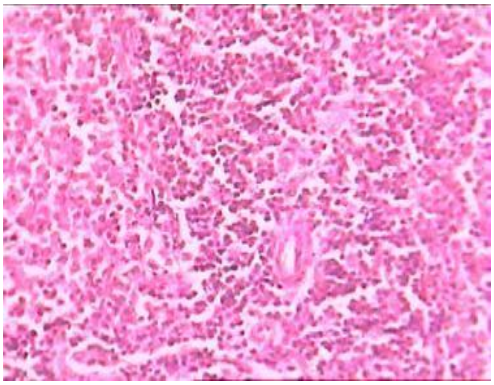
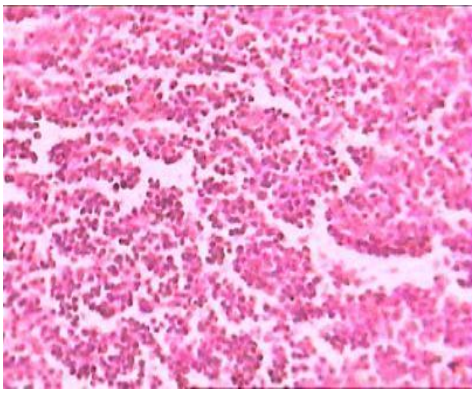
KIDNEY:

NORMAL **HIGH DOSE**

SPLEEN:

NORMAL **HIGH DOSE**

PHARMACOLOGICAL ACTIVITY

ANTI-TUBERCULOSIS ACTIVITY:

Table: 21 Anti- tuberculosis activity of *Thirijadhathi mathirai*

S.NO.	Compound Name	% Reduction in RLU		
		250µg/ml	500µg/ml	Rifampicin 2µg/ml
1.	*Mycobacterium tuberculosis $H_{37}Rv$*	52.15	56.98	93.66

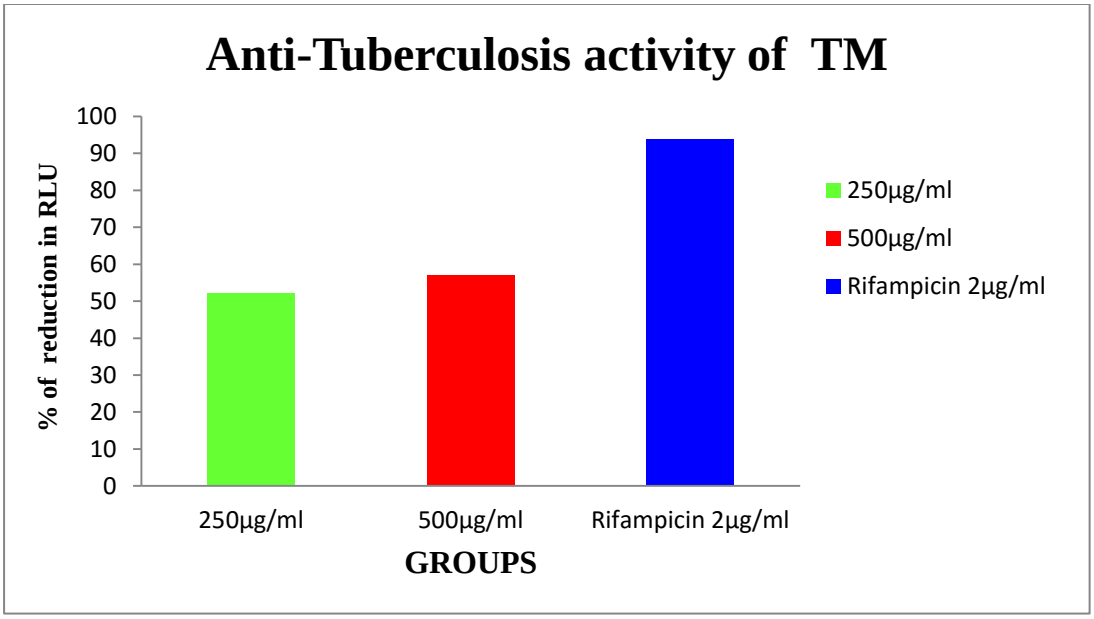


Chart 1: Anti tuberculosis activity

DISCUSSION:

When the TM was dissolve in DMSO, it is subjected into Luciferase reporter phage assay for anti-Mycobacterial activity study. The anti-Mycobacterial activity of TM is indicated by fifty five and above fifty five percent reductions in relative light units (RLU) in the presence of compound in comparison with compound free control. TM indicates anti- tuberculosis activity in *Mycobacterium tuberculosis $H_{37}Rv$*. From these above results we can conclude this Siddha herbal formulation TM validated as a valuable drug for the treatment of Tuberculosis.

BRONCHODILATOR ACTIVITY:

TABLE: 22 Bronchodilator activity of *Thirijadhathi mathirai*

Serial No	Group	Onset of Convulsion in sec.	% protection
1	Control	91.45±0.093	--
2	Standard Salbutamol 2 mg/kg	1032.0±4.5*	92.4
3	TM (200mg/kg)	254.4±1.96**	66.52
4	TM (400mg/kg)	406.2.±1.57**	79.12

*Values are mean ±S.E.M. (n=6) *p<0.01, **p<0.001compared with the control animals*

BRONCHODIALATOR ACTIVITY OF *THIRIJADHATHI MATHIRAI*

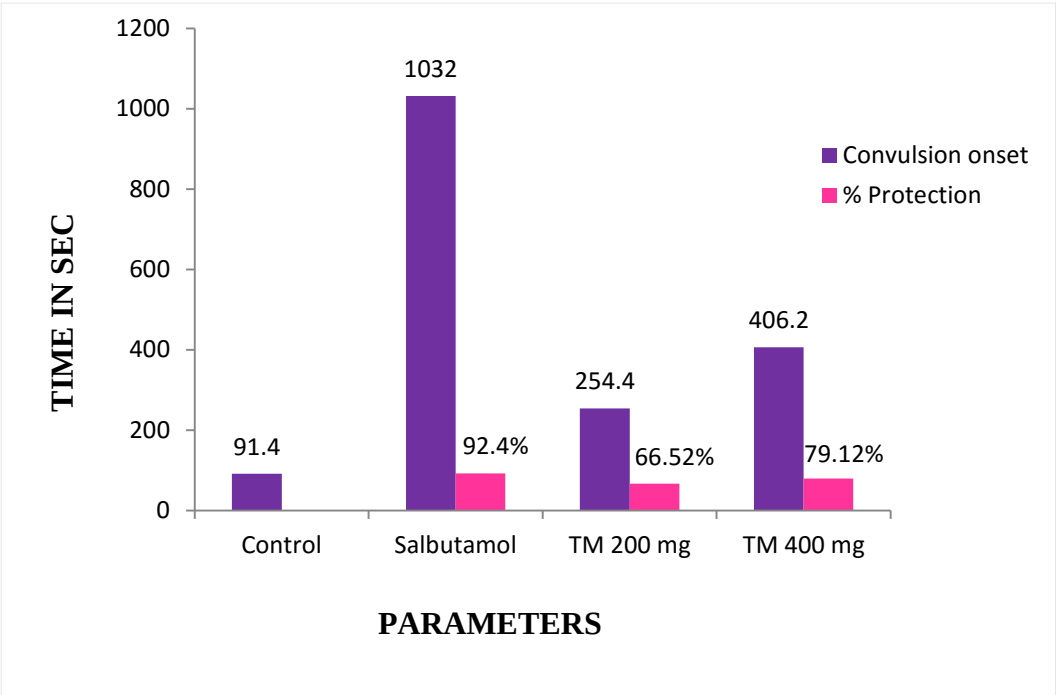


Chart 2: Bronchodilator activity

DISCUSSION:

From the above results, we can confirm that the *TM* possess bronchodilator activity nearer to fifty percentages when compared with Salbutamol as a standard drug.

ANTI-HISTAMINE ACTIVITY:

Table: 23 Anti-histamine effect of *Thirijadhathi mathirai*

S.no	Treatment Group	Mean exposition time insec After histamine exposure		Mean exposition time in sec after acetylcholine exposure	
		Before treatment	After treatment	Before treatment	After treatment
1	Normal	105.21±1.52	104.33±1.26	51.33±1.09	52.12±1.03
2	Chlorpheniramine	102.31±2.03	182.02±1.09	52.53±1.22	87.57±2.04
3	TM 200mg/kg	104.53±1.06	119.04±2.01	53.10±1.20	57.21±1.51
4	TM 400mg/kg	102.65±2.06	148.59±2.07	52.20±2.08	58.01±1.08

EXPOSITION TIME AFTER HISTAMINE AND ACETYLCHOLINE:

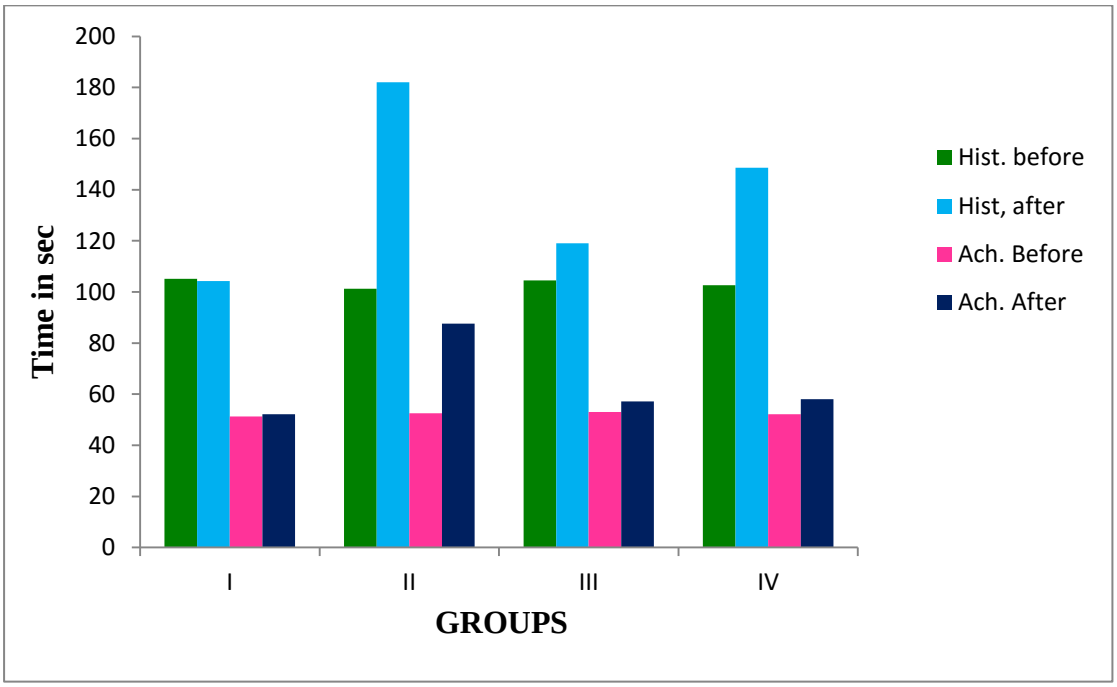


Chart 3: Anti-histamine activity

DISCUSSION

Mediators like histamine, serotonin and acetylcholine are implicated in various ways in the pathogenesis of Asthma. Histamine is the most implicated mediator in broncho constriction that accompanies asthma although the role of serotonin in asthma is uncertain. *Thirijadhathi mathirai* inhibited the histamine induced bronchospasm (vascular permeability) in rats, when compared with Chlorpheniramine as standard. Here, *Thirijadhathi mathirai* possess the Anti- histamine activity.

6. CONCLUSION

- The drug *Thirijadhathi mathirai* was selected from "*Anubava vaithiya devaragasiyam"* to validate the safety and its efficacy in treating for tuberculosis.
- The ingredients of the drug was identified and authenticated by Gunapadam experts. Then drug was prepared as per the literature and subjected to various studies.
- When it was subjected into physico chemical, organoleptic character it was fulfils all the parameters of *mathirai* according to AYUSH guidelines.
- In the phytochemical, biochemical analysis, HPTLC showed the presence of active ingredients responsible for its activity. Phyto chemical showed the presence of Alkaloids, Glycosides, Tannins, Phytosterol, Phenol, Triterpenes and Proteins. Biochemical analysis showed the presence of Potassium, Ammonium and phosphate. Thus, from those constituents it has a synergistic effect in acting against the disease.
- In instrumental analysis, FT-IR showed the peak values of functional groups present in the drug. SEM showed the particle size. And ICP-OES showed the presence of Calcium, Iron, Potassium, Sodium and Phosphorus and absence of cadmium, lead arsenic and mercury in the permissible limits.
- Toxicity studies done according to OECD guidelines showed the acute and repeated oral toxicity of the drug. The haematological and biochemical parameters showed slight various between 100, 200 and 400mg but within the limits when compared to control. The body weight of the animals has slight change (increased in body weight). Thus, it will establish the safety and potency of the drug when administrated for long time.
- *Thirijadhathi mathirai* could be conformed as no-observed-adverse effect level (NOAEL) drug as it acts harmless under normal usage and to be of no toxicological concern.
- Pharmacological studies was done In-vitro model for Anti-Mycobacterial activity and in animal model for bronchodilator and Anti-histamine activity.
- By using Luciferase reporter phage assay, the Anti-tuberculosis activity of TM had proved in mycobacterium tuberculosis $H_{37}Rv$.

- Bronchodilator activity, showed 50% of activity when compared with control in animal models.
- Anti-histamine activity done in vascular permeability test in rats models. The drug will attain above 50% of activity when compared with control.
- Thus, by scrutinizing all the above results, it is concluded that Anti tuberculosis, Anti-histamine and bronchodilator activity of TM was scientifically validated. Hence, it is safe and potent drug.

8. SUMMARY

In Siddha text "***Anubava vaidhya daeva ragasiyam***" the drug "*Thirijadhathi mathirai*" was selected for preclinical study to establish relevant strong literature indication to Anti tuberculosis, Bronchodilator and Anti-histamine activity.

The collection of relevant literature evidences used in the preparation of *Thirijadhathi mathirai* for the ingredients, which claims support as Anti-Mycobacterial agent.

The drug was subjected to various studies to prove its safety and efficacy.

Standardization by physico chemical, phytochemical analysis was done. Phytochemical analysis of the drug reveals the presence of alkaloids, tannins, glycosides and triterpenes which are responsible for Anti-Mycobacterial activity.

Instrumental analysis like FT-IR, SEM and ICP-OES are done. The finger print was engaged by using modern analytical technique like HPTLC. SEM analysis showed the sizes of the drug are nano and micro particle. ICP-OES showed the heavy metals are in BDL and or in permissible limits.

Toxicity of the drug was revealed in both acute and repeated oral toxicity studies, which proved the non-toxic and safety of the drug.

Pharmacological studies of the drug showed the Anti-tuberculosis activity by using Luciferase reporter phage assay has proved in both *Mycobacterium tuberculosis* $H_{37}Rv$ and clinical isolated S, H, R & E resistant. Hence, it can be concluded as a therapeutically effective drug in MDR-TB also.

The Bronchodilator and Anti-histamine activity of the drug *Thirijadhathi mathirai was* scientifically validated.

In conclusion, *Thirijadhathi mathirai* was evaluated to be non-toxic in the tested doses and suggested remarkable medicinal value in the treatment of tuberculosis.

The final discussion and conclusion analysed the dissertation. The most vital part of experiment of the findings is also discussed and thereafter it is implicate to future studies and future research if possible.

7. FUTURE SCOPE

In Luciferase reporter phage assay, the drug *Thirijadhathi mathirai* has its own potency against *Mycobacterium tuberculosis* $H_{37}Rv$ which has been established in this study.

An incredible action of this drug value against these organisms has been revealed from this study.

This must be implicated in the future chronic toxicity studies, preclinical studies in animal model and multi-centre clinical studies, which are required to understand the exact molecular mechanism of action. So it could be used to treat tuberculosis worldwide.

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The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....S. POONKUZHALI.....

For participating as ~~Resource~~ ~~Person~~ / Delegate in the Twenty Fourth Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 24th to 28th April 2017.

Dr.N.KABILAN, M.D.(S),Ph.D.,
PROF & HEAD DEPT.OF SIDDHA

Prof.**Dr.T.BALASUBRAMANIAN,** M.D.,D.L.O.,
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INSTITUTE OF SCIENCE AND TECHNOLOGY
CHENNAI – 600 119

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr./Ms~~. S. POONKUZHALI

of Govt. Siddha Medical College, Chennai has participated in the two-day workshop on "**TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS**" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

B. Sheela Rani

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R.S.

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T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

Dr. L. Lokoranjan
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Certificate of Achievement

This Certificate is proudly presented to

Dr. Poonkuzhali S
GSMC, Chennai

for making ~~oral presentation~~/poster presentation titled

A POLY-HERBAL FORMULATION OF MOOSAMBARA MEZHUGU FOR POLYCYSTIC OVARIAN SYNDROME

in the National Conference on "Prevention and Management of Lifestyle Disorders through Siddha system of Medicine" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

Prof. Dr. R. S. Ramaswamy
(Director General
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Certificate of Participation

This Certificate is proudly presented to

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for participating

in the National Conference on "*Prevention and Management of Lifestyle Disorders through Siddha system of Medicine*" on **the first Siddha Day** held on **04.01.2018** – organised by Central Council for Research in Siddha (CCRS) jointly with Directorate of Indian Medicine and Homoeopathy, Govt. of Tamil Nadu, The Tamil Nadu Dr. M.G.R. Medical University and National Institute of Siddha.

மார்கழி
ஆயில்யம்
சித்த மருத்துவத்
திருநாள்

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(Director General
Central Council for Research in Siddha)
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CERTIFICATE

This is to certify that Dr / Mrs / Miss / Mr. S. POONKUZHALI

has Participated in the CME on **"GOOD CLINICAL PRACTICE AND CLINICAL TRIALS"** conducted by the Department of Pharmacology, Govt. Stanley Medical College, Chennai on **04.07.2018 & 05.07.2018.**

Accredited by the Centre for Accreditation, The Tamilnadu Dr.MGR Medical University.

and has been awarded **10 credit points** under **Category II.**

DEAN
Govt. Stanley Medical College,
Chennai – 1.

Head of the Department
Department of Pharmacology
Govt. Stanley Medical College

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

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WORLD CONGRESS ON HOLISTIC HEALTH

Certificate of Participation

Dr/Mr/Mrs/Ms/ S. POONKUZHALI HAS PARTICIPATED IN THE WORLD CONGRESS ON HOLISTIC HEALTH ORGANISED BY THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY, CHENNAI ON 9TH & 10TH FEBRUARY 2018 AT SILVER JUBILEE AUDITORIUM.

CME CREDIT POINTS : 20

PROF. Dr. T.BALASUBRAMANIAN MS., DLO
REGISTRAR

PROF. Dr. S.GEETHALAKSHMI MD., Ph.D
VICE-CHANCELLOR

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Government Siddha Medical College

Arumbakkam, Chennai – 600 106

CERTIFICATE

Certified that the samples submitted for identification by S. Poonkuzhali PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients of *Thirijadhathi mathirai*:

1. *Lavangapattai (Cinnamomum verum)*
2. *Lavangapathiri (Cinnamomum tamala)*
3. *Elakkai (Eletteria cardamomum)*
4. *Thipilli (Piper longum)*
5. *Karkandu (Sugar candy)*
6. *Dried dhiratchai (Vitis vinifera)*
7. *Adhimathuram (Glycyrrhiza glabra)*
8. *Karchurkai (Phonex dactilifera)*

Date: 12.3.18

Place: Chennai

12/3/18

PG Department of Gunapadam

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All India Council for Technical Education, New Delhi

L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

APPROVAL CERTIFICATE

This is to certify that the project titled "SCIENTIFIC VALIDATION OF SIDDHA POLY-HERBAL FORMULATION OF ***"THIRIJADHATHI MATHIRAI"*** FOR ITS ANTI-TUBERCULOSIS, BRONCHO-DIALATOR AND ANTI-HISTAMINE ACTIVITY IN ANIMAL MODEL" has been approved by the 53rd IAEC.

IAEC no: 05/321/PO/Re/S/01/CPCSEA dated 12/10/2018

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

Dr.P.Muralidharan

(Member Secretary)